Molecular Human Reproduction, Vol.24, No.6 pp. 327–340, 2018
Advanced Access publication on April 2, 2018 doi:10.1093/molehr/gay013

molecular human reproduction

ORIGINAL ARTICLE

Critical role of mTOR, PPARγ and PPARδ signaling in regulating early pregnancy decidual function, embryo viability and feto-placental growth

Sabrina L. Roberti[1,2], Romina Higa[1,2], Verónica White[1,2], Theresa L. Powell[3,4], Thomas Jansson[4], and Alicia Jawerbaum[1,2,*]

[1]Universidad de Buenos Aires, Facultad de Medicina, Paraguay 2155, Buenos Aires, Argentina [2]CONICET-Universidad de Buenos Aires, Laboratory of Reproduction and Metabolism, CEFYBO, 1121 CABA, Buenos Aires, Argentina [3]Section of Neonatology, Department of Pediatrics, University of Colorado Denver Anschutz Medical Campus, Aurora, CO 80045, USA [4]Division of Reproductive Sciences, Department of OB/GYN, University of Colorado Denver Anschutz Medical Campus, Aurora, CO 80045, USA

*Correspondence address. Universidad de Buenos Aires, Facultad de Medicina and CONICET-Universidad de Buenos Aires, Laboratory of Reproduction and Metabolism, CEFYBO, 1121 CABA, Buenos Aires, Argentina. E-mail a.jawerbaum@gmail.com orcid.org/0000-0002-8456-2398

Submitted on November 24, 2017; resubmitted on February 28, 2018; editorial decision on March 7, 2018; accepted on March 8, 2018

STUDY QUESTION: What are the consequences of inhibiting mTOR, the mechanistic target of rapamycin (mTOR), and the peroxisome proliferator activated receptor gamma (PPARγ) and PPARδ pathways in the early post-implantation period on decidual function, embryo viability and feto-placental growth in the rat?

SUMMARY ANSWER: mTOR inhibition from Days 7 to 9 of pregnancy in rats caused decidual PPARγ and PPARδ upregulation on Day 9 of pregnancy and resulted in embryo resorption by Day 14 of pregnancy. PPARγ and PPARδ inhibition differentially affected decidual mTOR signaling and levels of target proteins relevant to lipid histotrophic nutrition and led to reduced feto-placental weights on Day 14 of pregnancy.

WHAT IS KNOWN ALREADY: Although mTOR, PPARγ and PPARδ are nutrient sensors important during implantation, the role of these signaling pathways in decidual function and how they interact in the early post-implantation period are unknown. Perilipin 2 (PLIN2) and fatty acid binding protein 4 (FABP4), two adipogenic proteins involved in lipid histotrophic nutrition, are targets of mTOR and PPAR signaling pathways in a variety of tissues.

STUDY DESIGN, SIZE, DURATION: Rapamycin (mTOR inhibitor, 0.75 mg/kg, sc), T0070907 (PPARγ inhibitor, 0.001 mg/kg, sc), GSK0660 (PPARδ inhibitor, 0.1 mg/kg, sc) or vehicle was injected daily to pregnant rats from Days 7 to 9 of pregnancy and the studies were performed on Day 9 of pregnancy ($n = 7$ per group) or Day 14 of pregnancy ($n = 7$ per group).

PARTICIPANTS/MATERIALS, SETTING, METHODS: On Day 9 of pregnancy, rat decidua were collected and prepared for western blot and immunohistochemical studies. On Day 14 of pregnancy, the resorption rate, number of viable fetuses, crown–rump length and placental and decidual weights were determined.

MAIN RESULTS AND THE ROLE OF CHANCE: Inhibition of mTOR in the early post-implantation period led to a reduction in FABP4 protein levels, an increase in PLIN2 levels and an upregulation of PPARγ and PPARδ in 9-day-pregnant rat decidua. Most embryos were viable on Day 9 of pregnancy but had resorbed by Day 14 of pregnancy. This denotes a key function of mTOR in the post-implantation period and suggests that activation of PPAR signaling was insufficient to compensate for impaired nutritional/survival signaling induced by mTOR inhibition. Inhibition of PPARγ signaling resulted in decreased decidual PLIN2 and FABP4 protein expression as well as in inhibition of decidual mTOR signaling in Day 9 of pregnancy. This treatment also reduced feto-placental growth on Day 14 of pregnancy, revealing the relevance of PPARγ signaling in sustaining post-implantation growth. Moreover, following inhibition of PPARδ, PLIN2 levels were decreased and mTOR complex 1 and 2 signaling was altered in decidua on Day 9 of pregnancy. On Day 14 of pregnancy, PPARδ inhibition caused reduced feto-placental weight, increased decidual weight and increased resorption rate, suggesting a key role of PPARδ in sustaining post-implantation development.

LARGE SCALE DATA: Not applicable.

LIMITATIONS, REASONS FOR CAUTION: This is an *in vivo* animal study and the relevance of the results for humans remains to be established.

WIDER IMPLICATIONS OF THE FINDINGS: The early post-implantation period is a critical window of development and changes in the intrauterine environment may cause embryo resorption and lead to placental and fetal growth restriction. mTOR, PPARγ and PPARδ signaling are decidual nutrient sensors with extensive cross-talk that regulates adipogenic proteins involved in histotrophic nutrition and important for embryo viability and early placental and fetal development and growth.

STUDY FUNDING/COMPETING INTEREST(S): Funding was provided by the Agencia Nacional de Promoción Científica y Tecnológica de Argentina (PICT 2014-411 and PICT 2015-0130), and by the International Cooperation (Grants CONICET-NIH-2014 and CONICET-NIH-2017) to A.J. and T.J. The authors have no conflicts of interest.

Key words: gestation / development / decidua / PPARs / histotrophic nutrition / lipids / embryo resorption / post-implantation period

Introduction

Emerging evidence suggests that the mechanistic target of rapamycin (mTOR) signaling functions as a placental nutrient sensor, regulating placental function and fetal growth in late pregnancy (Dimasuay *et al.*, 2016). Placental mTOR is activated in women with obesity or gestational diabetes mellitus (GDM) who deliver large babies and in animal models associated with fetal overgrowth (Gaccioli *et al.*, 2013; Jansson *et al.*, 2013; Capobianco *et al.*, 2016; Perez-Perez *et al.*, 2013). In contrast, intrauterine growth restriction (IUGR) is characterized by inhibition of placental mTOR signaling in humans (Roos *et al.*, 2007; Chen *et al.*, 2015) and in animal models (Rosario *et al.*, 2011; Kavitha *et al.*, 2014).

mTOR is a serine/threonine kinase that is activated by increased nutrient levels and growth factor signaling, thereby regulating gene transcription and protein translation to control multiple cellular processes that promote cell metabolism, growth and survival (Laplante and Sabatini, 2013; Shimobayashi and Hall, 2014). mTOR forms two protein complexes, mTORC1 and mTORC2, in association with raptor or rictor, respectively (Shimobayashi and Hall, 2014). Ribosomal protein S6 (rpS6) and 4E-binding protein 1 (4E-BP1) are downstream effectors of mTORC1 activation that positively regulate protein synthesis (Shimobayashi and Hall, 2014). 4E-BP1 phosphorylation induces the release of the eukaryotic initiation factor 4E (eIF4E), activating protein synthesis and cell growth (Laplante and Sabatini, 2013). mTORC2 phosphorylates a number of targets, including serum and glucocorticoid-inducible kinase 1 (SGK-1), with important roles in implantation (Lou *et al.*, 2017). Although mTOR signaling has been implicated in regulating implantation and trophoblast and embryo growth (Martin *et al.*, 2003; Gardner and Harvey, 2015), the function of mTOR signaling in the early post-implantation period remains to be determined.

In the post-implantation period, the decidua plays a fundamental nutritional role, providing histotrophic nutrition to allow embryo survival until the formation of a mature placenta (Burton *et al.*, 2010; Filant and Spencer, 2014). In murine embryos, the inverted yolk sac is crucial in histotrophic nutrition, as it is involved in the processes of endocytosis and pinocytosis of secretions from the uterine glands and decidual secretions (Holson *et al.*, 2005; Zohn and Sarkar, 2010). Lipoprotein particles are internalized in the yolk sac through the multiligand endocytic receptor complex for further repackage of lipids and transfer to the embryo (Herz and Farese, 1999; Zohn and Sarkar, 2010). The secretions from decidual cells and endometrial glands are rich in lipid droplets (Burton *et al.*, 2007). Lipid droplets are characterized by the presence of the adipogenic protein perilipin 2 (PLIN2), which is considered a marker for the lipid load of non-adipogenic cells (Heid *et al.*, 1998). PLIN2 is transcriptionally regulated by peroxisome proliferator activated receptor γ (PPARγ) and PPARδ in trophoblasts and other cell types (Bildirici *et al.*, 2003; Tobin *et al.*, 2006). In addition, fatty acid binding protein 4 (FABP4), a protein involved in intracellular and transcellular lipid transfer and relevant for decidual function (Tian *et al.*, 2011), is transcriptionally regulated by PPARγ (Tan *et al.*, 2002).

PPARs are transcription factors activated by ligands of lipid nature (e.g. mono- and polyunsaturated fatty acids) that function as master transcription factors to regulate gene expression in metabolic processes, critically important in maintaining lipid homeostasis and antioxidant/anti-inflammatory responses (Wahli and Michalik, 2012). Besides, they play relevant roles in embryo organogenesis and feto-placental development and metabolism (Jawerbaum and Capobianco, 2011; Kadam *et al.*, 2015; Lendvai *et al.*, 2016). There are three PPAR isoforms, and among which, PPARγ and PPARδ play key roles in adipogenesis and are essential for embryo development (Barak *et al.*, 2008; Barquissau *et al.*, 2017; Wahli and Michalik, 2012). Indeed, there are specific placental developmental defects in PPARγ and PPARδ null mice, which lead to embryo death, suggesting that both isoforms are essential and the absence of compensatory effects by other PPAR isoforms that rescue the adverse phenotype (Wang *et al.*, 2007; Barak *et al.*, 2008). Our previous studies showed reduced embryonic PPARδ and decidual PPARγ in diabetic rats during early organogenesis, changes associated with impaired embryo morphogenesis (Higa *et al.*, 2010, 2014). Moreover, changes in fatty acid metabolism involve extensive PPAR and mTOR cross-talk in a variety of cell types (Ishiguro *et al.*, 2006; Blanchard *et al.*, 2012; Angela *et al.*, 2016) and these nutrient signaling pathways share targets such as FABP4 (Wang *et al.*, 2017). In the current study, we hypothesized that there is a cross-talk between mTOR, PPARγ and PPARδ in the decidua that impacts post-implantation embryo survival and feto-placental growth. To test this hypothesis, we determined the effects of pharmacological inhibition of mTOR, PPARγ and PPARδ signaling during the early post-implantation period in pregnant rats.

Materials and Methods

Animals

Albino Wistar rats were obtained from the certified animal facility of the School of Exact and Natural Sciences, University of Buenos Aires (UBA, Argentina). In our animal facility (CEFYBO-CONICET, UBA, Argentina), rats received water and food *ad libitum* (commercial rat chow, Asociación Cooperativa Argentina, Buenos Aires, Argentina), on a lighting cycle of

12 h light:12 h dark. The animal protocol was approved by the Institutional Committee for the Care and Use of Experimental Animals (CICUAL, Resolution CD No. 1497/2013), School of Medicine, University of Buenos Aires, and conducted according to the Guide for the Care and Use of Laboratory Animals, US National Institutes of Health (NIH Publication, 8th edition, 2011) http://www.ncbi.nlm.nih.gov/books/NBK54050/?report=reader. Females were mated and mating was confirmed by the presence of sperm in vaginal smears and this day was designated Day 1 of pregnancy. Pregnant females received three daily sc injections beginning on Day 7 of pregnancy: receiving either (i) rapamycin (mTOR inhibitor, 0.75 mg/kg, LC Laboratories, Massachusetts, USA); (ii) T0070907 (PPARγ inhibitor, 0.001 mg/kg, Sigma Aldrich, Missouri, USA); (iii) GSK0660 (PPARδ inhibitor, 0.1 mg/kg, Sigma Aldrich, Missouri, USA) or (iv) vehicle (control). The doses of the inhibitors used were chosen based on bibliography and preliminary experiments (Lee *et al.*, 2002; Harston *et al.*, 2011; Tsai *et al.*, 2014). The dosing interval was chosen aiming to address an early and presomite embryo post-implantation stage in which the decidua is crucial for embryo development and nutrition. Half of the animals were euthanized in a CO_2 chamber on Day 9 of pregnancy (3 h after the last injection) and the remainder on Day 14 of pregnancy. Using a stereomicroscope and microsurgical dissecting instruments, the balls of decidual tissue were explanted from each uterus on Day 9 of pregnancy ($n = 7$ dams in each experimental group). The decidua were prepared for immunohistochemical analyses of the levels of FABP4 and PLIN2, or homogenized in ice-cold buffer D (250 mM sucrose, 10 mM Hepes-Tris, pH 7.4) with protease inhibitor cocktail and phosphatase inhibitor cocktails 2 and 3 (Sigma Aldrich, Missouri, USA) and stored at −80°C for western blot analysis of PPARγ, PPARδ and functional readouts of the mTOR pathway. Decidua, fetuses and placentas from 14-day pregnant rats ($n = 7$ dams in each experimental group) were explanted from each uterus, examined morphologically under a stereomicroscope and weighed.

Immunohistochemistry

Immunostaining of FABP4 and PLIN2 was performed in the decidua of 9-day pregnant rats ($n = 7$ in each experimental group). Each decidua was paraffinized and serially sectioned (5 μm). Sections were deparaffinized, rehydrated through a graded series of ethanol and the endogenous peroxidase activity was blocked. The sections were processed using the corresponding primary antibodies: anti-FABP4 (rabbit polyclonal antibody (GTX54016), 1:100 dilution, GeneTex, CA, USA), and anti-PLIN-2 (mouse monoclonal antibody (LS-C348703 clone AP125), 1:100 dilution, LifeSpan BioSciences, Washington, USA). All primary antibodies were checked for antibody specificity by Western blot and diluted in PBS-Tween with 1% bovine serum albumin (BSA). Negative controls were performed in the absence of primary antibody and by replacing the primary antibody by a pooled serum of the same species that contains a spectrum of the IgG subclasses (Vector Laboratories, Burlingame, CA, USA). Biotinylated antibodies (anti-rabbit IgG or anti-mouse IgG-rat absorbed), 1:200 PBS-Tween in 1.5% normal horse serum (Vector Laboratories, Burlingame, CA, USA), were applied, followed by an incubation with an avidin–biotin complex for 60 min (Vector Laboratories). Staining was visualized by adding 40% *w/v* 3,3′-diaminobenzidine tetrahydrochloride chromogen-buffer plus 0.02% (*v/v*) hydrogen peroxide in 0.05 M Tris (pH 7.6) for 10 min (Martinez *et al.*, 2012). Three entire sections per decidua were examined using light microscopy by two skilled blinded observers. Immunoreactivity intensity was quantified using the ImageProPlus software. Data are shown as relative to a value of 1, arbitrarily assigned to the control. We also performed a semiquantitative score, which led to similar results (data not shown). In addition, also in the yolk sac and the uterine glands a semiquantitative score was performed.

Western blot analysis

Proteins from homogenates of pooled decidua from the same litter ($n = 7$ rats in each experimental group) were separated by SDS-PAGE and transferred to PVDF membranes (35 V constant, overnight at 4°C), as previously described (Capobianco *et al.*, 2016). The membranes were stained with Ponceau S staining solution for total proteins (Sigma-Aldrich) to confirm equal loading and transfer. Blocking was carried out for 1 h at room temperature in 1% BSA in TBS-Tween and membranes were incubated with the primary antibody (diluted in 1% BSA in TBS-Tween) overnight at 4°C. Protein expression of total and phosphorylated rpS6, total and phosphorylated 4E-BP1 was determined using antibodies (monoclonal 2217 clone 5G10 and polyclonal 2211, 9452 and 9459, respectively) from Cell Signaling Technology (Massachusetts, USA). Total and phosphorylated SGK-1 was evaluated using polyclonal antibodies (AF3200) from RyD Systems (Minnesota, USA) and (SC16745) from Santa Cruz (Texas, USA), respectively. PLIN2 was determined using antibodies from LifeSpan BioSciences (LS-C348703 clone AP125), while protein expression of PPARγ and PPARδ was determined using polyclonal antibodies (101 700 and 101 720, respectively) from Cayman Chemical Company, Michigan, USA. After washing, the membranes were incubated with the appropriate peroxidase conjugated secondary antibody and visualized using ECL detection solution (Thermo Scientific) and captured in a Chemiluminescence imaging system (GeneGnomeXRQ, Syngene). Densitometry analysis was performed with ImageJ software (Schneider *et al.*, 2012). The expression of the target protein in each individual lane was normalized for total protein staining to adjust for unequal loading. The mean of all the samples within a single gel was calculated and the expression of the target protein in each sample was calculated as a percentage of that mean (target/total protein).

Statistical analysis

Data are presented as the mean ± SEM. Groups were compared by Student *t* test or Chi-square test as appropriate. A $P < 0.05$ was considered statistically significant.

Results

Effects of inhibition of mTOR signaling in the early post-implantation period

Aiming to address the impact of mTOR inhibition limited to an early post-implantation period, rapamycin (0.75 mg/kg, sc) was administered to pregnant rats on Days 7, 8 and 9 of pregnancy and the decidua were evaluated 3 h after the last injection on Day 9 of pregnancy. Rapamycin administration led to more than 50% reduction in the phosphorylation of downstream targets of mTORC1 (rpS6 and 4E-BP1) and mTORC2 (SGK-1) in the decidua (Supplementary Fig. S1). We determined the protein expression of FABP4 and found that the inhibition of mTOR signaling resulted in a 22% reduction of FABP4 levels in the decidua ($P < 0.05$), when compared to control rats (Fig. 1). A similar pattern of expression was observed in decidual cells and in the uterine glands, although only a weak staining, which did not change with the rapamycin treatment, was observed in the yolk sac (Fig. 1). We next evaluated the protein expression of PLIN2 and found that the inhibition of mTOR signaling resulted in a 25% increase in decidual PLIN2 levels ($P < 0.01$) when compared to controls (Fig. 1). This increase was also observed when PLIN2 protein

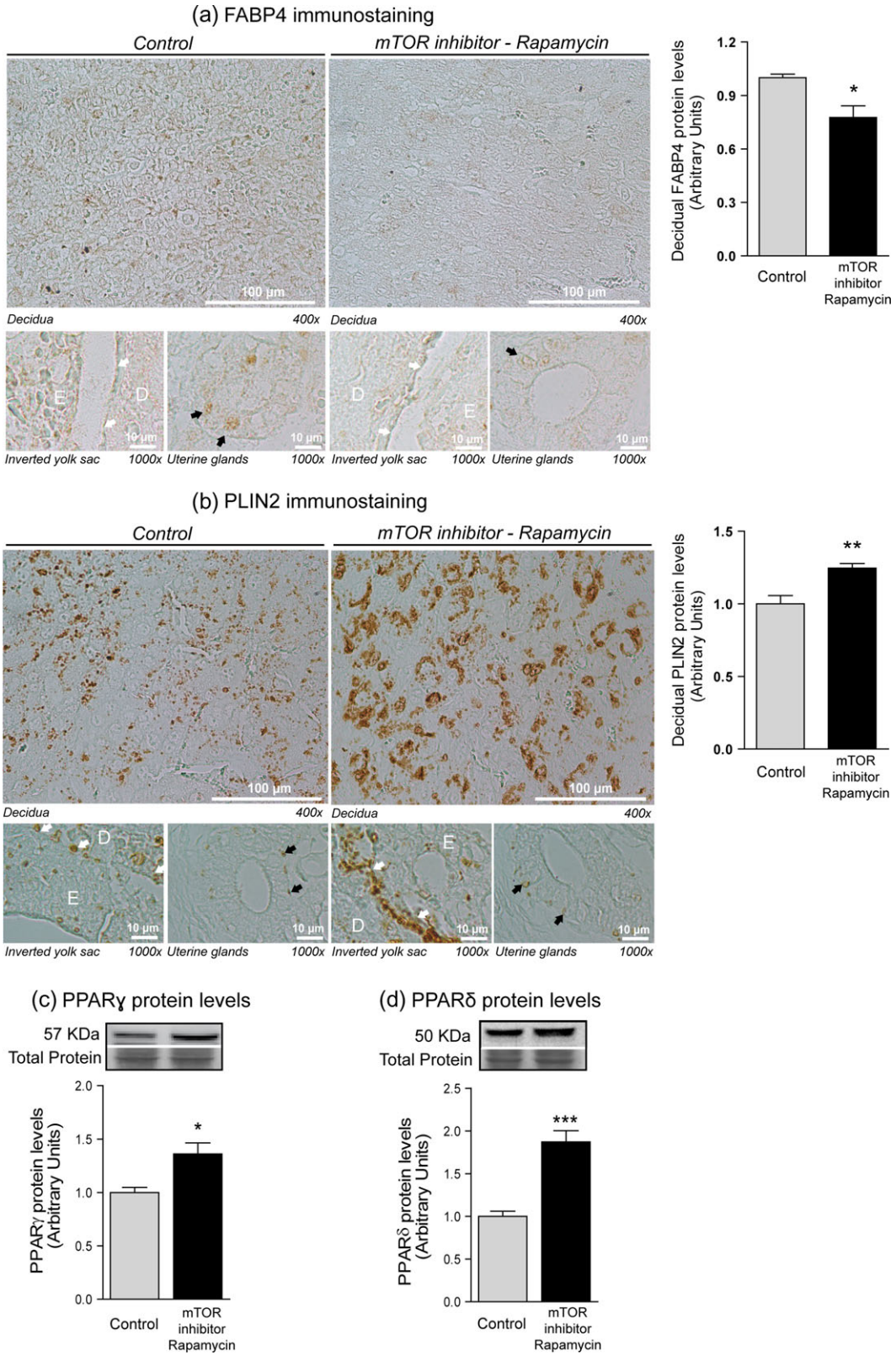

Figure 1 Effect of *in vivo* treatments with the mTOR inhibitor rapamycin during Days 7–9 of pregnancy on decidual protein levels of (**a**) FABP4, (**b**) PLIN2, (**c**) PPARγ and (**d**) PPARδ, evaluated on Day 9 of pregnancy. Values represent mean ± SEM obtained from seven rats in each experimental group. Statistical analysis: Student *t* test. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus control. Representative photos of FABP4 and PLIN2 in the decidua, yolk sac and uterine glands are shown. D, decidua. E, embryo. White arrows point to the yolk sac. Black arrows point to immunostained uterine gland cells.

levels were determined by western blot analysis (Supplementary Fig. S2). Different from decidual cells, only a weak PLIN2 staining was observed in the uterine glands, although the yolk sac showed a strong PLIN2 staining, which increases when mTOR signaling was inhibited (Fig. 1). As PLIN2 is a classical PPAR target involved in the formation of lipid droplets (Heid *et al.*, 1998; Bildirici *et al.*, 2003), and mTOR and PPAR cross-talk has been described in different tissues/cell types (Blanchard *et al.*, 2012; Angela *et al.*, 2016), we next measured the protein expression of PPARγ and PPARδ. We observed that mTOR inhibition caused an increase in the protein levels of both PPARγ (36%, $P < 0.05$) and PPARδ (87%, $P < 0.001$) in the decidua (Fig. 1).

Effects of inhibition of PPARγ signaling in the early post-implantation period

To next address the effect of PPARγ inhibition limited to an early post-implantation stage, the PPARγ inhibitor T0070907 (0.001 mg/kg, sc) was administered to rats on Days 7–9 of pregnancy and the decidua were evaluated on Day 9 of pregnancy. PPARγ inhibition led to a decrease in the protein levels of FABP4 (40%) in the decidua when compared to controls ($P < 0.01$, Fig. 2). A reduced FABP4 expression was also observed in the uterine glands, although only a weak staining was observed in the yolk sac in both the control and the PPARγ inhibited groups (Fig. 2). The inhibition of PPARγ led to a decrease in the

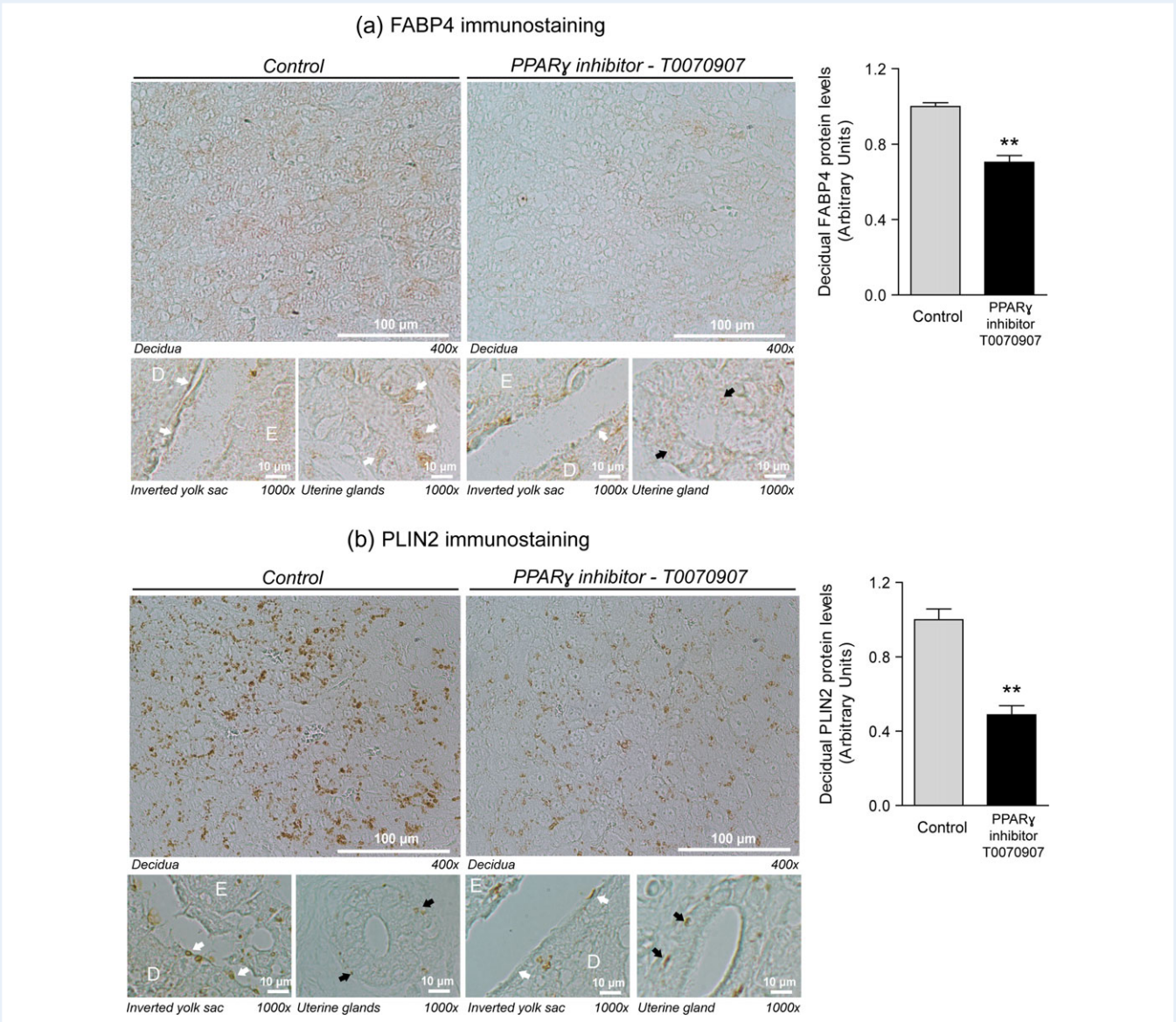

Figure 2 Effect of *in vivo* treatments with the PPARγ inhibitor T0070907 during Days 7–9 of pregnancy on decidual protein levels of (**a**) FABP4 and (**b**) PLIN2, evaluated on Day 9 of pregnancy. Values represent mean ± SEM obtained from seven rats in each experimental group. Statistical analysis: Student *t* test. $^{**}P < 0.01$ versus control. Representative photos of FABP4 and PLIN2 in the decidua, yolk sac and uterine glands are shown. D, decidua. E, embryo. White arrows point to the yolk sac. Black arrows point to immunostained uterine gland cells.

protein levels of PLIN2 (51%) in the decidua when compared to controls ($P < 0.01$, Fig. 2). A decrease was also evidenced when decidual PLIN2 protein levels were determined by western blot analysis (Supplementary Fig. S2). In contrast to decidual cells, only a weak PLIN2 staining was observed in the uterine glands from the control and the PPARγ inhibited group. Similarly to decidual cells, PLIN2 levels were reduced in the yolk sac when PPARγ was inhibited (Fig. 2). In addition, PPARγ inhibition decreased the phosphorylation of rpS6 ($P < 0.01$) by a 25% without affecting total rpS6 levels in the decidua, whereas it did not change the levels of total and phosphorylated 4E-BP1 compared to controls (Fig. 3). Moreover, we observed a marked reduction of phosphorylated SGK-1 (50%, $P < 0.01$), despite unchanged total SGK-1 levels in the decidua from T0070907-treated pregnant rats compared to controls (Fig. 3). Collectively, these data demonstrate that inhibition of decidual PPARγ inhibits mTORC1 and mTORC2 signaling.

Effects of inhibition of PPARδ signaling in the early post-implantation period

To address the effect of PPARδ inhibition limited to an early post-implantation period, the PPARδ inhibitor GSK0660 (0.1 mg/kg, sc) was administered to rats on days 7 to 9 of pregnancy and the decidua were evaluated on Day 9 of pregnancy. PPARδ inhibition did not alter decidual FABP4 protein levels compared to controls (Fig. 4). Similarly, PPARδ inhibition did not change FABP4 levels in the uterine glands (Fig. 4). Only a weak FABP4 staining was detected in the yolk sac from rats treated or not with the PPARδ inhibitor (Fig. 4). The inhibition of PPARδ led to a decrease in the protein levels of PLIN2 (37%) in the decidua when compared to controls ($P < 0.01$, Fig. 4). A similar decrease was evidenced when decidual PLIN2 levels were determined by western blot analysis (Supplementary Fig. S2). In the uterine glands, only a weak PLIN2 staining was observed in both the control and the PPARδ inhibited groups (Fig. 4). Differently, the yolk sac showed a strong PLIN2 staining, which diminished when PPARδ was inhibited, similarly to that observed in decidual cells (Fig. 4).

With respect to cross-talk with mTOR pathways, PPARδ inhibition did not affect the total expression or phosphorylation of rpS6 in the decidua, but markedly increased 4E-BP1 phosphorylation by a 73% ($P < 0.001$, Fig. 5) compared to controls, without affecting the total levels of this protein, suggesting the activation of this branch of the mTORC1 signaling pathway. In contrast, decidual levels of phosphorylated SGK-1 were found to be reduced by 53% ($P < 0.001$, Fig. 5) following PPARδ inhibition, whereas no changes in the levels of total SGK-1 were observed, suggesting the inhibition of the mTORC2 signaling pathway.

Effects of mTOR and PPAR signaling inhibition on resorption rates and growth capacity

The number of viable embryos and the resorption rate on Day 9 of pregnancy were similar in the control rats and in the rats that received the mTOR inhibitor (rapamycin 0.75 mg/kg, sc) from Days 7 to 9 of pregnancy (Fig. 6). To further assess the effects of mTOR inhibition in the early post-implantation period on embryo viability and growth capacity, we evaluated the conceptus on Day 14 of pregnancy following administration of vehicle (control) or the mTOR inhibitor (rapamycin 0.75 mg/kg) from Days 7 to 9 of pregnancy. On Day 14 of pregnancy, a dramatic (92%) decrease in the number of viable fetuses per rat ($P < 0.001$) and an increased resorption rate ($P < 0.001$) were evident in the group that received the mTOR inhibitor compared to controls (Fig. 6). Indeed, most fetuses were resorbed, and the few fetuses that survived to Day 14 of pregnancy displayed morphological

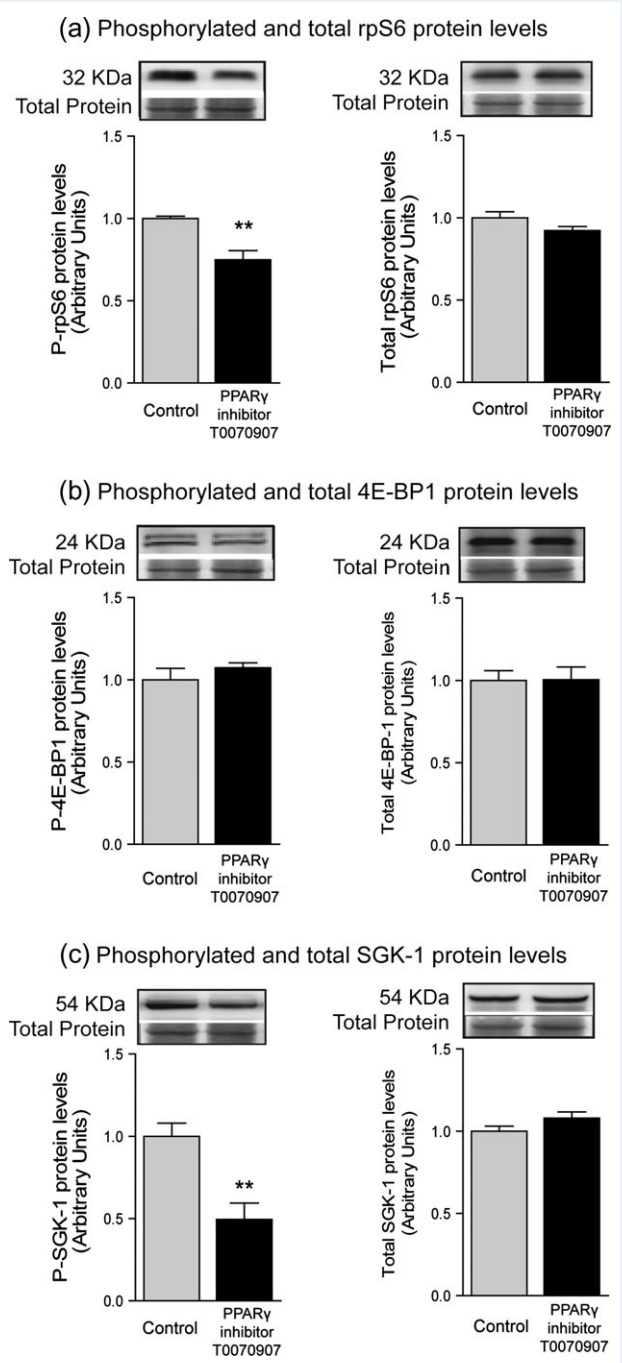

Figure 3 Effect of *in vivo* treatments with the PPARγ inhibitor T0070907 during Days 7–9 of pregnancy on decidual protein levels of (**a**) phosphorylated and total rpS6, (**b**) phosphorylated and total 4E-BP1 and (**c**) phosphorylated and total SGK-1, evaluated on Day 9 of pregnancy. Values represent mean ± SEM obtained from seven rats in each experimental group. Statistical analysis: Student *t* test. $^{**}P < 0.01$ versus control.

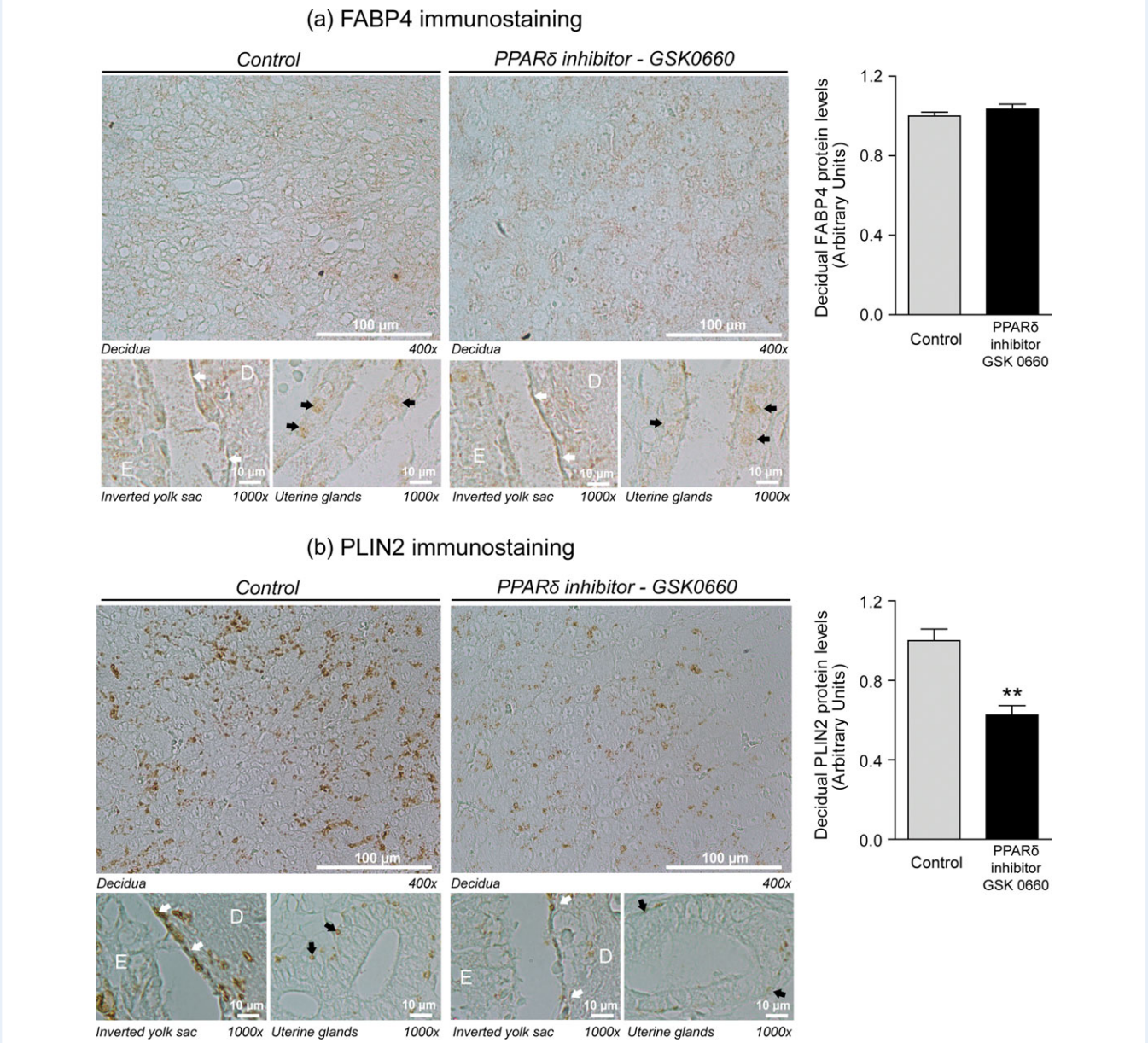

Figure 4 Effect of *in vivo* treatments with the PPARδ inhibitor GSK0660 during Days 7–9 of pregnancy on decidual protein levels of (**a**) FABP4 and (**b**) PLIN2, evaluated on Day 9 of pregnancy. Values represent mean ± SEM obtained from seven rats in each experimental group. Statistical analysis: Student *t* test. $^{**}P < 0.01$ versus Control. Representative photos of FABP4 and PLIN2 in the decidua, yolk sac and uterine glands are shown. D, decidua. E, embryo. White arrows point to the yolk sac. Black arrows point to immunostained uterine gland cells.

alterations and a severe developmental delay likely leading to future resorptions (Fig. 7).

In the rats that received the PPARγ inhibitor (T0070907 0.001 mg/kg, sc) from Days 7 to 9 of pregnancy, the number of viable embryos and the resorption rate on Day 9 of pregnancy were similar to those of control rats and remained similar on Day 14 of pregnancy (Fig. 8). The inhibition of PPARγ did not affect gross fetal morphology on Day 14 of pregnancy (Fig. 7), but the reduced crown–rump length ($P < 0.001$) (Fig. 8) indicated a growth delay. In addition, placental weight was markedly reduced (51%, $P < 0.001$) following PPARγ inhibition, although decidual weight was unchanged when compared to controls (Fig. 8).

In the rats that received the PPARδ inhibitor (GSK0660 0.1 mg/kg, sc) from Days 7 to 9 of pregnancy, the number of viable embryos and the resorption rate on Day 9 of pregnancy were similar to those of control rats (Fig. 9). However, on Day 14 of pregnancy, the rats that received the PPARδ inhibitor during the early post-implantation period showed a reduction in the number of viable fetuses ($P < 0.05$) and an increased resorption rate ($P < 0.01$) as compared to controls (Fig. 9). Although there were no gross malformations on the 14-day fetuses in the group that received the PPARδ inhibitor (Fig. 7), they were growth restricted, as indicated by their reduced crown–rump length ($P < 0.01$) (Fig. 9). Furthermore, PPARδ inhibition was associated with

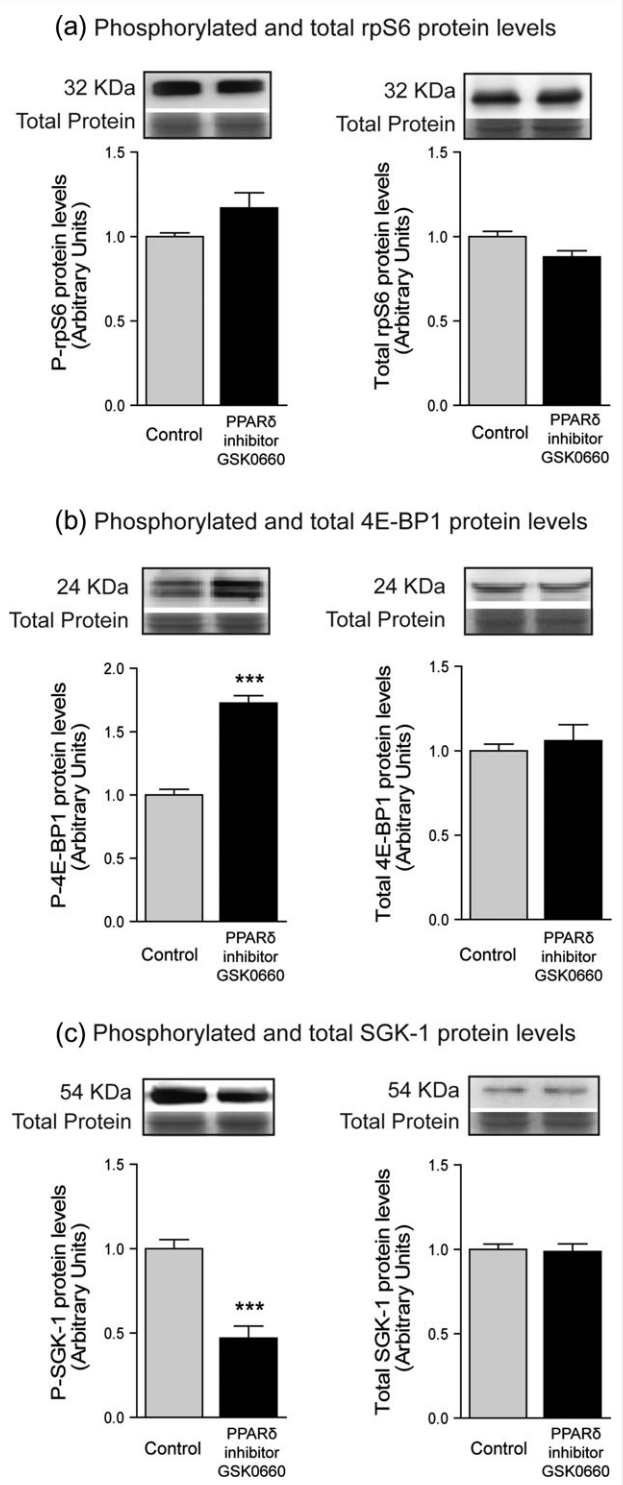

Figure 5 Effect of *in vivo* treatments with the PPARδ inhibitor GSK0660 during Days 7 to 9 of pregnancy on decidual protein levels of (**a**) phosphorylated and total rpS6, (**b**) phosphorylated and total 4E-BP1 and (**c**) phosphorylated and total SGK-1, evaluated on Day 9 of pregnancy. Values represent mean ± SEM obtained from seven rats in each experimental group. Statistical analysis: Student *t* test. ***$P < 0.001$ versus control.

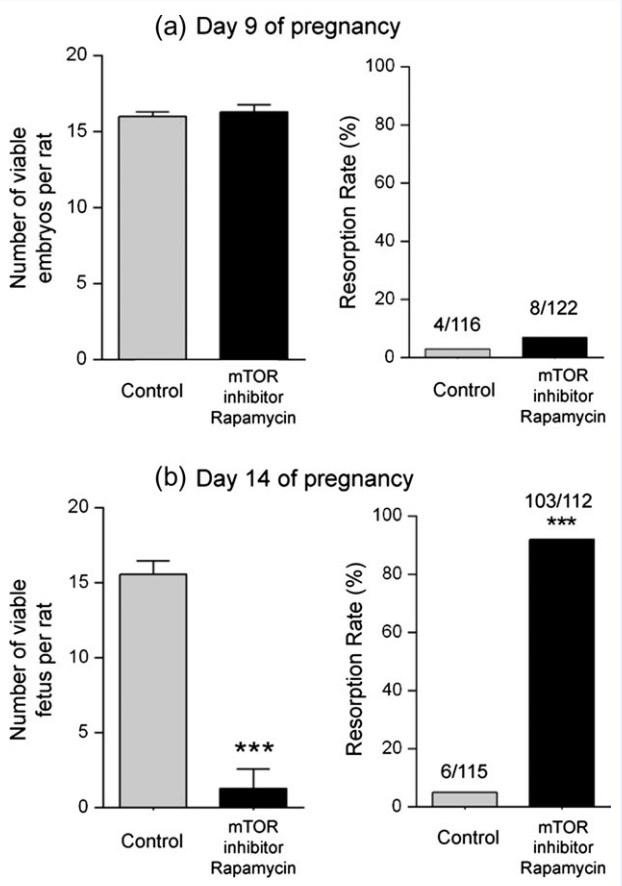

Figure 6 Effect of *in vivo* treatments with the mTOR inhibitor rapamycin during Days 7–9 of pregnancy on the number of viable embryos per rat and the resorption rate (**a**) on Day 9 of pregnancy and (**b**) on Day 14 of pregnancy. Values represent mean ± SEM obtained from seven rats in each experimental group. Statistical analysis: Student *t* test or Chi-square test (percentage of resorption data). ***$P < 0.001$ versus control.

reduced placental weight (35%, $P < 0.01$), and increased decidual weight on Day 14 of pregnancy (35%, $P < 0.001$) (Fig. 9).

Discussion

Following implantation and before the mature placenta is established, the embryo relies on histotrophic nutrition through secretions from decidual cells and endometrial glands in both humans and rodents (Burton *et al.*, 2010; Filant and Spencer, 2014). In rodents, the inverted yolk sac favors the absorption of nutrients (Zohn and Sarkar, 2010). Although the role of placental mTOR and PPARs as nutrient sensors in late pregnancy is established, the function of these signaling pathways in regulating histotrophic nutrition in the early post-implantation period is largely unknown. We report for the first time that when mTOR, PPARγ or PPARδ pathways are inhibited in the early post-implantation period, a complex cross-talk between these signaling pathways is observed in the decidua, leading to reduced levels of adipogenic proteins relevant for histotrophic nutrition. Specifically, although inhibition

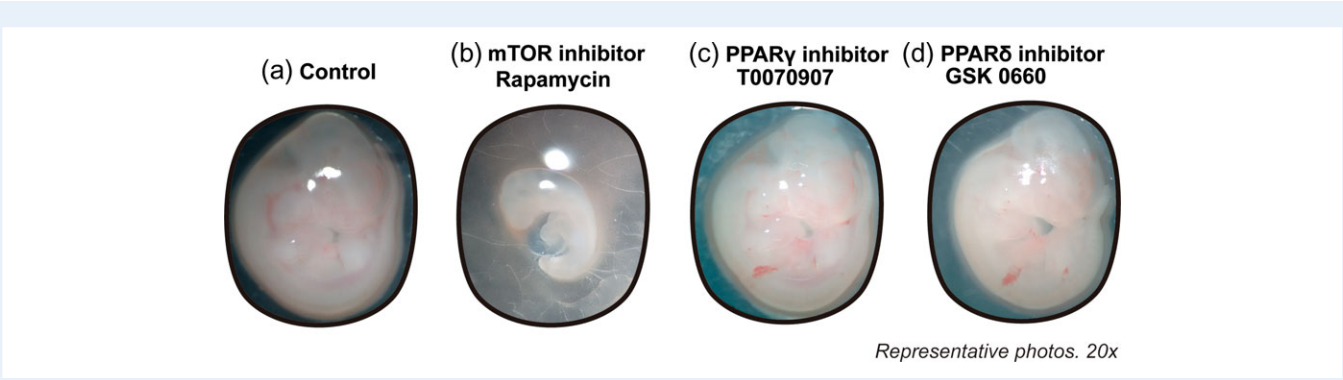

Figure 7 Representative figures of fetuses obtained from 14-day pregnant rats that received (**a**) vehicle, (**b**) the mTOR inhibitor rapamycin, (**c**) the PPARγ inhibitor T0070907 and (**d**) the PPARδ inhibitor GSK0660 from Days 7 to 9 of pregnancy.

of these nutrient sensors does not affect early post-implantation embryo viability, it leads to embryo loss, growth delay or placental/decidual growth impairments in mid-gestation.

There is a growing interest in understanding the first trimester of human pregnancy, not only to improve the understanding of implantation failure and miscarriage but also in the search of possible early causes of pregnancy complications that typically are diagnosed later in pregnancy, including gestational diabetes and IUGR (Albu *et al.*, 2014; Kennelly and McAuliffe, 2016). The first trimester of pregnancy is characterized by histotrophic nutrition, which provides essential nutrients to the growing embryo and allows a proper placental development (Burton *et al.*, 2010; Filant and Spencer, 2014). To address the impact of nutrient sensors in the early post-implantation period, we administered mTOR, PPARγ and PPARδ inhibitors on Days 7–9 of rat pregnancy, a developmental stage corresponding to the very early post-implantation embryonic and placental development that occurs in the second week of human pregnancy. Our data reveal the importance of this early post-implantation period in determining fetal growth and identify distinct effects of the inhibition of mTOR, PPARγ or PPARδ signaling on decidual proteins relevant for histotrophic nutrition.

The inhibition of mTOR limited to this short post-implantation period had a pronounced impact on mid-gestation outcomes. Indeed, the resorption rate on Day 14 of pregnancy was extremely high and occurred despite the observed upregulation of PPARγ and PPARδ in the decidua, which may be compensatory. The cross-talk between mTOR and PPARs seems to be dependent on the tissue and cell type, as it is well described that in adipose tissue, mTOR activation stimulates PPARγ-induced lipid uptake and fat accretion (Kim and Chen, 2004; Blanchard *et al.*, 2012). The increased abundance of PLIN2 protein in the decidua is likely to be the result of PPARγ activation, given the well established role of PPARγ as a transcriptional regulator of PLIN2 in trophoblasts and other cell types (Bildirici *et al.*, 2003). In this study, PLIN2 levels were low in the uterine glands but high in the yolk sac, which show a further increase in their levels when mTOR is inhibited, suggesting a common regulation in the decidual cells and the yolk sac.

mTOR inhibition caused a decrease in FABP4 protein expression, in both decidua and uterine glands, similarly to that observed in other cell types (Wang *et al.*, 2017), and despite PPARγ activation, which has been reported to positively regulate FABP4 in different tissues (Tan *et al.*, 2002).

Although it is well established that IUGR is associated with inhibition of placental mTORC1 and mTORC2 signaling (Roos *et al.*, 2007; Rosario *et al.*, 2011; Kavitha *et al.*, 2014; Chen *et al.*, 2015), the severity of the phenotype observed in this work demonstrates that the early post-implantation period is highly susceptible to mTOR inhibition, implicating mTOR as a nutrient sensing and growth promoting signaling pathway critical for normal development and survival at this developmental stage. mTOR is important for the growth and survival of the trophoblast and embryo prior to implantation as evidenced by the lack of viability of mTOR null mice (Murakami *et al.*, 2004), and impaired trophoblast outgrowth following mTOR inhibition in pre-implantation embryos in culture (Martin *et al.*, 2003). These reports, together with the findings in the present study, are consistent with a critical role of mTOR in embryo viability from conception to the early post-implantation stage. In addition to the well-known role of mTOR modulating placental nutrient transport and mitochondrial respiration and thereby regulating fetal growth (Kavitha *et al.*, 2014), a recent study in which rapamycin was given to rats during late pregnancy has shown that mTOR signaling is involved in the programming of heart defects in the offspring (Hennig *et al.*, 2017). Moreover, placental mTOR signaling activity in IUGR correlates with growth during the first year of life (Fahlbusch *et al.*, 2015). Therefore, mTOR signaling appears to play an important role in determining growth and survival from early pregnancy to the postnatal period.

In the present study, a less severe phenotype was observed when PPARγ was inhibited in the early post-implantation period in the rat. Our results are consistent with a role for PPARγ as a nutrient sensor, capable of regulating the levels of FABP4 in the decidua and uterine glands and the levels of PLIN2 in the decidua and yolk sac, adipogenic proteins involved in cellular and tissue lipid metabolism (Heid *et al.*, 1998; Li *et al.*, 2018; Scifres *et al.*, 2011). As FABP4 is involved in decidualization and PLIN2 modulates lipid accumulation and transfer in different tissues, including the placenta (Bildirici *et al.*, 2003; Scifres *et al.*, 2011; Frank *et al.*, 2015; Tian *et al.*, 2011; Li *et al.*, 2018), the downregulation of these adipogenic proteins in the decidua, uterine glands and yolk sac may be directly linked to the feto-placental growth restriction observed following PPARγ inhibition. The proposed histotrophic and growth promoting role of these adipogenic proteins in the decidua is supported by the reported metabolic and histotrophic role of PLIN2 in tumor cell growth and the ability of FABP4 to regulate proliferation,

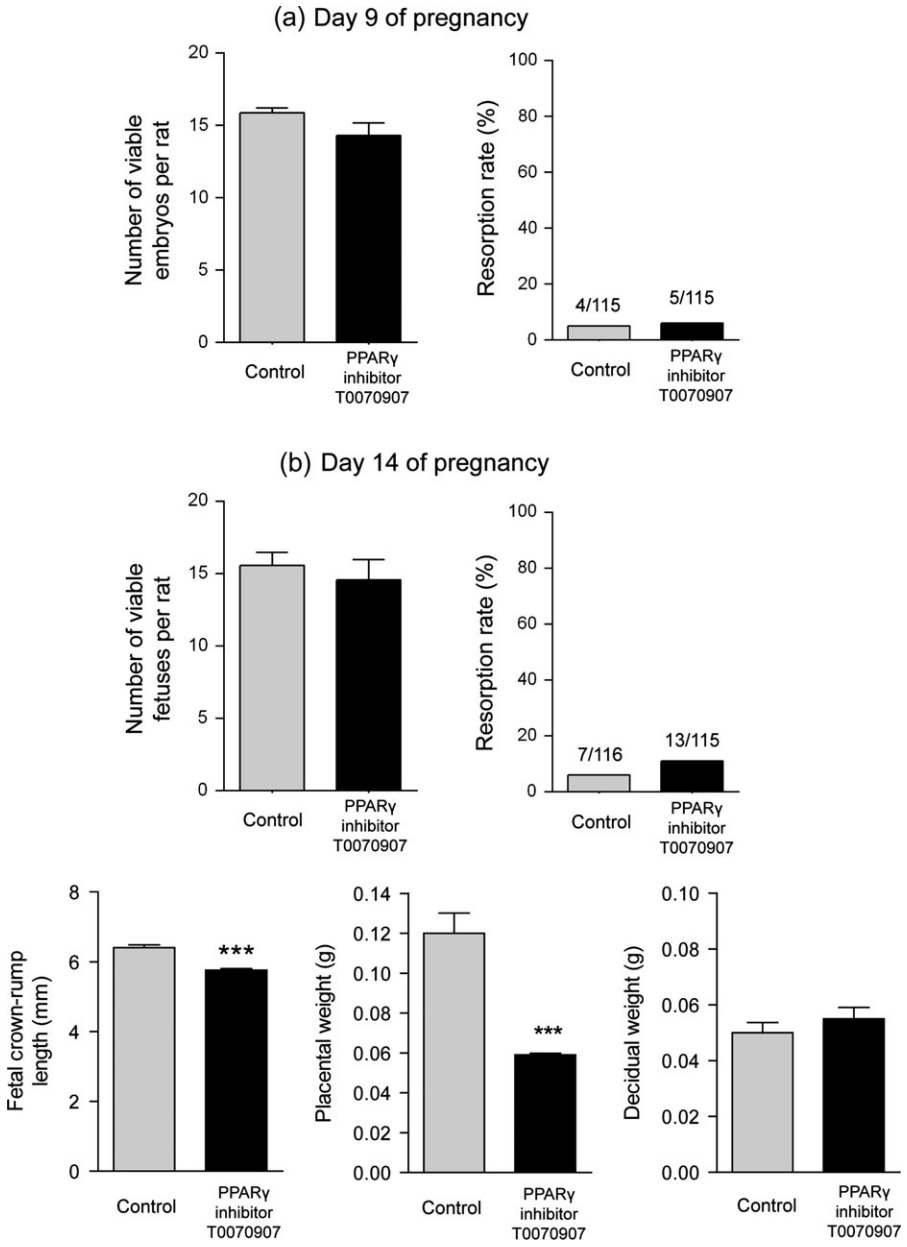

Figure 8 Effect of *in vivo* treatments with the PPARγ inhibitor T0070907 during Days 7 to 9 of pregnancy on (**a**) the number of viable embryos per rat and the resorption rate on Day 9 of pregnancy and (**b**) the number of viable fetuses per rat, the resorption rate, the fetal crown–rump length and the placental and decidual weight on Day 14 of pregnancy. Values represent mean ± SEM obtained from seven rats in each experimental group. Statistical analysis: Student *t* test or Chi-square test (percentage of resorption data). ***$P < 0.001$ versus control.

migration and invasion of human uterine endometrial epithelial cells (Zhu *et al.*, 2015; Koizume and Miyagi, 2016).

Although diverse studies have shown that PPARγ activation negatively regulates mTOR signaling in different tissues (San *et al.*, 2015; Osman and Segar, 2016; Capobianco *et al.*, 2017), by studying the decidua, we found that the activities of both mTORC1 and mTORC2 signaling pathways were reduced when the rats were treated with the PPARγ inhibitor. Despite this reduction, there was no increased resorption rate on Day 14 of pregnancy. The different outcomes in pregnancies in which mTOR was inhibited using rapamycin (causing a high resorption rate) and in pregnancies in which PPARγ was inhibited (associated with mTOR inhibition but no effect on resorption rates) may be related to a less pronounced inhibition of mTOR in rats following PPARγ inhibition as compared to rats treated with the mTOR inhibitor.

In rats treated with the PPARγ inhibitor during the early post-implantation period, both the reduction in levels of adipogenic proteins, which is expected to limit lipid transfer to the developing embryo (Bildirici *et al.*, 2003; Scifres *et al.*, 2011), and the reduced mTOR signaling, expected to inhibit transfer of amino acids and cell growth

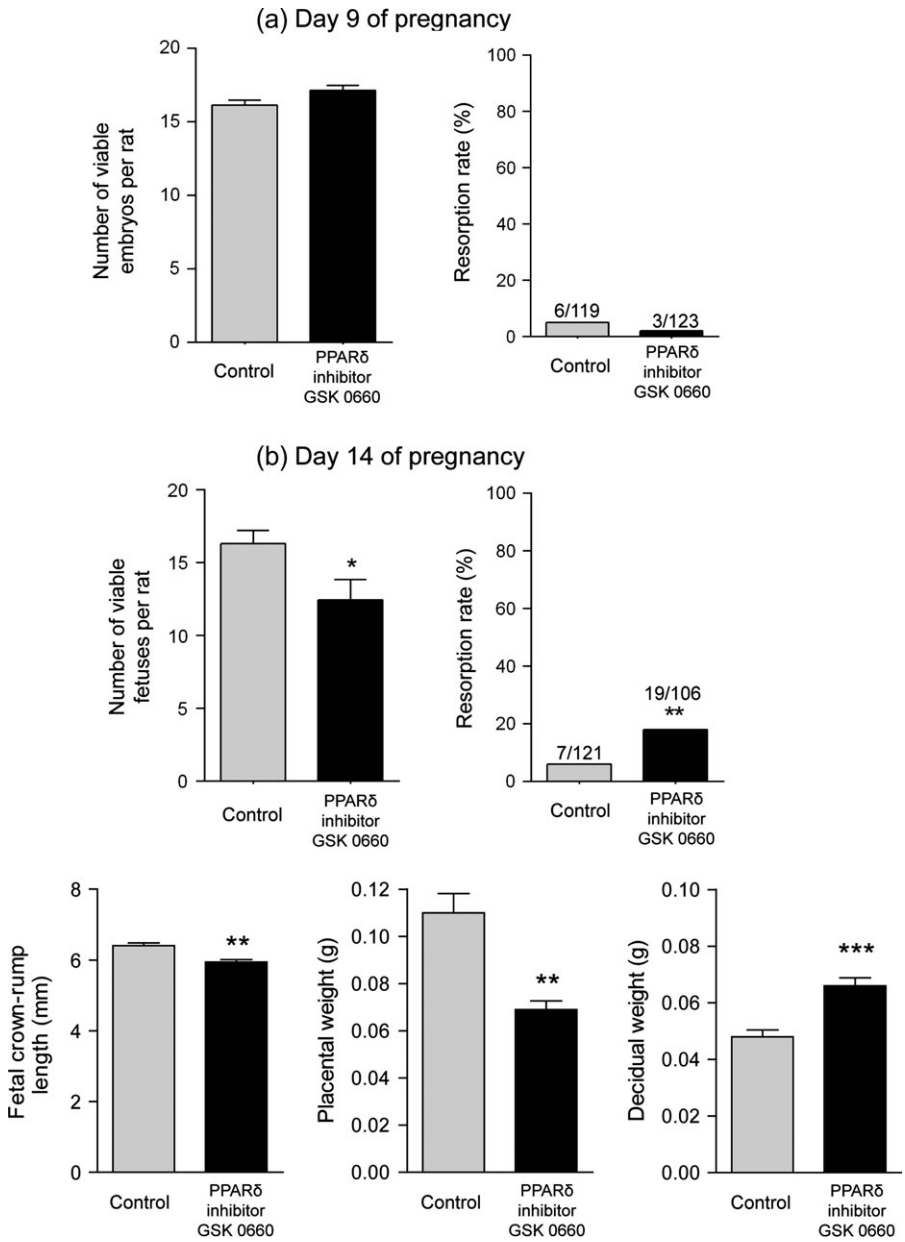

Figure 9 Effect of *in vivo* treatments with the PPARδ inhibitor GSK0660 during Days 7–9 of pregnancy on (**a**) the number of viable embryos per rat and the resorption rate on Day 9 of pregnancy and (**b**) the number of viable fetuses per rat, the resorption rate, the fetal crown–rump length and the placental and decidual weight on Day 14 of pregnancy. Values represent mean ± SEM obtained from seven rats in each experimental group. Statistical analysis: Student *t* test or Chi-square test (percentage of resorption data). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus control.

(Shimobayashi and Hall, 2014; Dimasuay *et al.*, 2016), may be involved in the reduced fetal and placental weight evidenced in the post-placentation period. The mechanisms linking the inhibition of critical nutrient sensing signaling pathways on Days 7–9 of gestation to the outcomes on Day 14 of pregnancy remain to be established but may involve epigenetic mechanisms or effects on stem/progenitor cell populations. Indeed, PPAR and mTOR signaling regulates epigenetic and stem cell differentiation in different tissues/cell types, leading to developmental and metabolic changes (Meyer *et al.*, 2016; Vadla and Haldar, 2018).

PPARδ inhibition in pregnant rats in the early post-implantation period caused no changes in FABP4 levels in the decidua and uterine glands, but resulted in a reduction in PLIN2 protein levels in the decidua and yolk sac, which may contribute to an impaired lipid histotrophic nutrition. To the best of our knowledge, PLIN2 has not been previously studied in the context of decidual function, but our data are consistent with the possibility that PLIN2 is regulated by mTOR and PPAR nutrient sensing pathways and is involved in histotrophic nutrition in early pregnancy. Indeed, the strong PLIN2 immunostaining evident in the inverted yolk sac suggests its contribution to the various

mechanisms described in the yolk sac that allow a proper transfer of lipids to the developing murine embryos (Herz and Farese, 1999; Zohn and Sarkar, 2010).

PPARδ inhibition on Days 7–9 of pregnancy resulted in increased decidual levels of phosphorylated 4E-BP1, a branch of the mTORC1 pathway that promotes initiation of protein translation, thus leading to increased protein synthesis. It is possible that these changes contribute to the decidual overgrowth observed in this experimental group. On the other hand, decidual mTORC2 signaling was inhibited following PPARδ inhibition, which, together with the reduction of decidual PLIN2 levels, may have detrimental effects and lead to the reduced feto-placental growth evidenced on Day 14 of pregnancy. Moreover, embryo resorptions were also observed in this experimental group, highlighting an important role of PPARδ in the decidua in the early post-implantation period. These observations extend to the early post-implantation period the previously established critical role of PPARδ in implantation (Wang *et al.*, 2007).

Our results point to an impaired nutritional effect in the decidua, which leads to the adverse results observed in embryo and placental development. Nevertheless, as a limitation of this work, it is unknown whether some of these effects are secondary to systemic (endocrine) effects in the mother. Moreover, as PPARs and mTOR pathways are involved in angiogenic pathways in the placenta and other tissues/cell types (Wang *et al.*, 2007; Jiang and Liu, 2008), a possible regulation of angiogenic pathways that may be involved in the observed effects deserves further research. Finally, further studies addressing a rescue of the phenotype through mTOR and PPARs activation will be needed to improve the understanding of mTOR and PPAR signaling in embryo organogenesis.

In conclusion, our findings are consistent with an important role of decidual mTOR and PPAR nutrient sensing pathways in a very early post-implantation period. Possibly by regulating histotrophic nutrition, these nutrient sensors determine embryo viability and the growth of the placenta and the fetus.

Supplementary data

Supplementary data are available at *Molecular Human Reproduction* online.

Acknowledgements

The authors would like to thank Vet. Marcela Márquez and Tech. Enzo Cuba for the valuable help with animal handling.

Authors' role

S.R., R.H. and V.W. performed the experiments and contributed to the analysis and interpretation of the data. T.P. and T.J. contributed to the analysis and interpretation of the data and critically revised the article. A. J. designed the study, contributed to the analysis and interpretation of the data and drafted the article. All the authors contributed to the construction of this article and approved the final version to be published.

Funding

Agencia Nacional de Promoción Científica y Tecnológica de Argentina (PICT 2014-411 and PICT 2015-0130), and by the International Cooperation (Grants CONICET-NIH-2014 and CONICET-NIH-2017) to A.J. and T.J.

Conflicts of interest

None.

References

Albu AR, Anca AF, Horhoianu VV, Horhoianu IA. Predictive factors for intrauterine growth restriction. *J Med Life* 2014;**7**:165–171.

Angela M, Endo Y, Asou HK, Yamamoto T, Tumes DJ, Tokuyama H, Yokote K, Nakayama T. Fatty acid metabolic reprogramming via mTOR-mediated inductions of PPARgamma directs early activation of T cells. *Nat Commun* 2016;**7**:13683.

Barak Y, Sadovsky Y, Shalom-Barak T. PPAR signaling in placental development and function. *PPAR Res* 2008;**2008**:142082.

Barquissau V, Ghandour RA, Ailhaud G, Klingenspor M, Langin D, Amri EZ, Pisani DF. Control of adipogenesis by oxylipins, GPCRs and PPARs. *Biochimie* 2017;**136**:3–11.

Bildirici I, Roh CR, Schaiff WT, Lewkowski BM, Nelson DM, Sadovsky Y. The lipid droplet-associated protein adipophilin is expressed in human trophoblasts and is regulated by peroxisomal proliferator-activated receptor-gamma/retinoid X receptor. *J Clin Endocrinol Metab* 2003;**88**: 6056–6062.

Blanchard PG, Festuccia WT, Houde VP, St-Pierre P, Brule S, Turcotte V, Cote M, Bellmann K, Marette A, Deshaies Y. Major involvement of mTOR in the PPARgamma-induced stimulation of adipose tissue lipid uptake and fat accretion. *J Lipid Res* 2012;**53**:1117–1125.

Burton GJ, Jauniaux E, Charnock-Jones DS. Human early placental development: potential roles of the endometrial glands. *Placenta* 2007;**28**: S64–S69.

Burton GJ, Jauniaux E, Charnock-Jones DS. The influence of the intrauterine environment on human placental development. *Int J Dev Biol* 2010; **54**:303–312.

Capobianco E, Fornes D, Linenberg I, Powell TL, Jansson T, Jawerbaum A. A novel rat model of gestational diabetes induced by intrauterine programming is associated with alterations in placental signaling and fetal overgrowth. *Mol Cell Endocrinol* 2016;**422**:221–232.

Capobianco E, Fornes D, Roberti SL, Powell TL, Jansson T, Jawerbaum A. Supplementation with polyunsaturated fatty acids in pregnant rats with mild diabetes normalizes placental PPARgamma and mTOR signaling in female offspring developing gestational diabetes. *J Nutr Biochem* 2017; **53**:39–47.

Chen YY, Rosario FJ, Shehab MA, Powell TL, Gupta MB, Jansson T. Increased ubiquitination and reduced plasma membrane trafficking of placental amino acid transporter SNAT-2 in human IUGR. *Clin Sci (Lond)* 2015;**129**:1131–1141.

Dimasuay KG, Boeuf P, Powell TL, Jansson T. Placental responses to changes in the maternal environment determine fetal growth. *Front Physiol* 2016;**7**:12.

Fahlbusch FB, Hartner A, Menendez-Castro C, Nogel SC, Marek I, Beckmann MW, Schleussner E, Ruebner M, Huebner H, Dorr HG *et al*. The placental mTOR-pathway: correlation with early growth trajectories following intrauterine growth restriction? *J Dev Orig Health Dis* 2015; **6**:317–326.

Filant J, Spencer TE. Uterine glands: biological roles in conceptus implantation, uterine receptivity and decidualization. *Int J Dev Biol* 2014;**58**: 107–116.

Frank DN, Bales ES, Monks J, Jackman MJ, MacLean PS, Ir D, Robertson CE, Orlicky DJ, McManaman JL. Perilipin-2 modulates lipid absorption and microbiome responses in the mouse intestine. *PLoS One* 2015;**10**: e0131944.

Gaccioli F, White V, Capobianco E, Powell TL, Jawerbaum A, Jansson T. Maternal overweight induced by a diet with high content of saturated fat activates placental mTOR and eIF2alpha signaling and increases fetal growth in rats. *Biol Reprod* 2013;**89**:96.

Gardner DK, Harvey AJ. Blastocyst metabolism. *Reprod Fertil Dev* 2015;**27**: 638–654.

Harston RK, McKillop JC, Moschella PC, Van Laer A, Quinones LS, Baicu CF, Balasubramanian S, Zile MR, Kuppuswamy D. Rapamycin treatment augments both protein ubiquitination and Akt activation in pressure-overloaded rat myocardium. *Am J Physiol Heart Circ Physiol* 2011;**300**: H1696–H1706.

Heid HW, Moll R, Schwetlick I, Rackwitz HR, Keenan TW. Adipophilin is a specific marker of lipid accumulation in diverse cell types and diseases. *Cell Tissue Res* 1998;**294**:309–321.

Hennig M, Fiedler S, Jux C, Thierfelder L, Drenckhahn JD. Prenatal mechanistic target of rapamycin complex 1 (m TORC1) inhibition by rapamycin treatment of pregnant mice causes intrauterine growth restriction and alters postnatal cardiac growth, morphology, and function. *J Am Heart Assoc* 2017;**6**:pii:e005506. doi:10.1161/JAHA.117.005506.

Herz J, Farese RV Jr. The LDL receptor gene family, apolipoprotein B and cholesterol in embryonic development. *J Nutr* 1999;**129**:473S–475S.

Higa R, Roberti SL, Musikant D, Mazzucco MB, White V, Jawerbaum A. Effects of maternal dietary olive oil on pathways involved in diabetic embryopathy. *Reprod Toxicol* 2014;**49C**:185–195.

Higa R, White V, Martinez N, Kurtz M, Capobianco E, Jawerbaum A. Safflower and olive oil dietary treatments rescue aberrant embryonic arachidonic acid and nitric oxide metabolism and prevent diabetic embryopathy in rats. *Mol Hum Reprod* 2010;**16**:286–295.

Holson JF, Stump DG, Pearce LB, Watson RE, DeSesso JM. Mode of action: yolk sac poisoning and impeded histiotrophic nutrition—HBOC-related congenital malformations. *Crit Rev Toxicol* 2005;**35**: 739–745.

Ishiguro H, Katano Y, Nakano I, Ishigami M, Hayashi K, Honda T, Goto H, Bajotto G, Maeda K, Shimomura Y. Clofibrate treatment promotes branched-chain amino acid catabolism and decreases the phosphorylation state of mTOR, eIF4E-BP1, and S6K1 in rat liver. *Life Sci* 2006;**79**: 737–743.

Jansson N, Rosario FJ, Gaccioli F, Lager S, Jones HN, Roos S, Jansson T, Powell TL. Activation of placental mTOR signaling and amino acid transporters in obese women giving birth to large babies. *J Clin Endocrinol Metab* 2013;**98**:105–113.

Jawerbaum A, Capobianco E. Review: effects of PPAR activation in the placenta and the fetus: implications in maternal diabetes. *Placenta* 2011;**32**: S212–S217.

Jiang BH, Liu LZ. Role of mTOR in anticancer drug resistance: perspectives for improved drug treatment. *Drug Resist Updat* 2008;**11**:63–76.

Kadam L, Kohan-Ghadr HR, Drewlo S. The balancing act—PPAR-gamma's roles at the maternal-fetal interface. *Syst Biol Reprod Med* 2015;**61**:65–71.

Kavitha JV, Rosario FJ, Nijland MJ, McDonald TJ, Wu G, Kanai Y, Powell TL, Nathanielsz PW, Jansson T. Down-regulation of placental mTOR, insulin/IGF-I signaling, and nutrient transporters in response to maternal nutrient restriction in the baboon. *FASEB J* 2014;**28**:1294–1305.

Kennelly MA, McAuliffe FM. Prediction and prevention of Gestational Diabetes: an update of recent literature. *Eur J Obstet Gynecol Reprod Biol* 2016;**202**:92–98.

Kim JE, Chen J. Regulation of peroxisome proliferator-activated receptor-gamma activity by mammalian target of rapamycin and amino acids in adipogenesis. *Diabetes* 2004;**53**:2748–2756.

Koizume S, Miyagi Y. Lipid droplets: a key cellular organelle associated with cancer cell survival under normoxia and hypoxia. *Int J Mol Sci* 2016;**17**:pii: E1430. doi:10.3390/ijms17091430.

Laplante M, Sabatini DM. Regulation of mTORC1 and its impact on gene expression at a glance. *J Cell Sci* 2013;**126**:1713–1719.

Lee G, Elwood F, McNally J, Weiszmann J, Lindstrom M, Amaral K, Nakamura M, Miao S, Cao P, Learned RM *et al*. T0070907, a selective ligand for peroxisome proliferator-activated receptor gamma, functions as an antagonist of biochemical and cellular activities. *J Biol Chem* 2002; **277**:19649–19657.

Lendvai A, Deutsch MJ, Plosch T, Ensenauer R. The peroxisome proliferator-activated receptors under epigenetic control in placental metabolism and fetal development. *Am J Physiol Endocrinol Metab* 2016; **310**:E797–E810.

Li J, Mihalcioiu M, Li L, Zakikhani M, Camirand A, Kremer R. Vitamin D prevents lipid accumulation in murine muscle through regulation of PPARgamma and perilipin-2 expression. *J Steroid Biochem Mol Biol* 2018; **177**:116–124.

Lou Y, Hu M, Mao L, Zheng Y, Jin F. Involvement of serum glucocorticoid-regulated kinase 1 in reproductive success. *FASEB J* 2017;**31**:447–456.

Martin PM, Sutherland AE, Van Winkle LJ. Amino acid transport regulates blastocyst implantation. *Biol Reprod* 2003;**69**:1101–1108.

Martinez N, Sosa M, Higa R, Fornes D, Capobianco E, Jawerbaum A. Dietary treatments enriched in olive and safflower oils regulate seric and placental matrix metalloproteinases in maternal diabetes. *Placenta* 2012; **33**:8–16.

Meyer MB, Benkusky NA, Sen B, Rubin J, Pike JW. Epigenetic plasticity drives adipogenic and osteogenic differentiation of marrow-derived mesenchymal stem cells. *J Biol Chem* 2016;**291**:17829–17847.

Murakami M, Ichisaka T, Maeda M, Oshiro N, Hara K, Edenhofer F, Kiyama H, Yonezawa K, Yamanaka S. mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. *Mol Cell Biol* 2004;**24**:6710–6718.

Osman I, Segar L. Pioglitazone, a PPARgamma agonist, attenuates PDGF-induced vascular smooth muscle cell proliferation through AMPK-dependent and AMPK-independent inhibition of mTOR/p70S6K and ERK signaling. *Biochem Pharmacol* 2016;**101**:54–70.

Perez-Perez A, Maymo JL, Gambino YP, Guadix P, Duenas JL, Varone CL, Sanchez-Margalet V. Activated translation signaling in placenta from pregnant women with gestational diabetes mellitus: possible role of leptin. *Horm Metab Res* 2013;**45**:436–442.

Roos S, Jansson N, Palmberg I, Saljo K, Powell TL, Jansson T. Mammalian target of rapamycin in the human placenta regulates leucine transport and is down-regulated in restricted fetal growth. *J Physiol* 2007;**582**: 449–459.

Rosario FJ, Jansson N, Kanai Y, Prasad PD, Powell TL, Jansson T. Maternal protein restriction in the rat inhibits placental insulin, mTOR, and STAT3 signaling and down-regulates placental amino acid transporters. *Endocrinology* 2011;**152**:1119–1129.

San YZ, Liu Y, Zhang Y, Shi PP, Zhu YL. Peroxisome proliferator-activated receptor-gamma agonist inhibits the mammalian target of rapamycin signaling pathway and has a protective effect in a rat model of status epilepticus. *Mol Med Rep* 2015;**12**:1877–1883.

Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012;**9**:671–675.

Scifres CM, Chen B, Nelson DM, Sadovsky Y. Fatty acid binding protein 4 regulates intracellular lipid accumulation in human trophoblasts. *J Clin Endocrinol Metab* 2011;**96**:E1083–E1091.

Shimobayashi M, Hall MN. Making new contacts: the mTOR network in metabolism and signalling crosstalk. *Nat Rev Mol Cell Biol* 2014;**15**:155–162.

Tan NS, Shaw NS, Vinckenbosch N, Liu P, Yasmin R, Desvergne B, Wahli W, Noy N. Selective cooperation between fatty acid binding proteins

and peroxisome proliferator-activated receptors in regulating transcription. *Mol Cell Biol* 2002;**22**:5114–5127.

Tian Z, Zhao ZA, Liang XH, Zhang XH, Sha AG, Zhang ZR, Yu YS, Yang ZM. Expression and function of fatty acid-binding protein 4 during mouse decidualization. *Fertil Steril* 2011;**95**:2749–2752 e2741-2745.

Tobin KA, Harsem NK, Dalen KT, Staff AC, Nebb HI, Duttaroy AK. Regulation of ADRP expression by long-chain polyunsaturated fatty acids in BeWo cells, a human placental choriocarcinoma cell line. *J Lipid Res* 2006;**47**:815–823.

Tsai CC, Lee KS, Chen SH, Chen LJ, Liu KF, Cheng JT. Decrease of PPARdelta in type-1-like diabetic rat for higher mortality after spinal cord injury. *PPAR Res* 2014;**2014**:456386.

Vadla R, Haldar D. Mammalian target of rapamycin complex 2 (mTORC2) controls glycolytic gene expression by regulating Histone H3 Lysine 56 acetylation. *Cell Cycle* 2018;**17**:110–123.

Wahli W, Michalik L. PPARs at the crossroads of lipid signaling and inflammation. *Trends Endocrinol Metab* 2012;**23**:351–363.

Wang Y, Chen J, Tang W, Zhang Y, Li X. Rapamycin inhibits the proliferation of endothelial cells in hemangioma by blocking the mTOR-FABP4 pathway. *Biomed Pharmacother* 2017;**85**:272–279.

Wang H, Xie H, Sun X, Tranguch S, Zhang H, Jia X, Wang D, Das SK, Desvergne B, Wahli W *et al*. Stage-specific integration of maternal and embryonic peroxisome proliferator-activated receptor delta signaling is critical to pregnancy success. *J Biol Chem* 2007;**282**:37770–37782.

Zhu Q, Jin Y, Wang P, Wang H, Lu B, Wang Z, Dong M. Expression and function of fatty acid-binding protein 4 in epithelial cell of uterine endometrium. *Cell Biol Int* 2015;**39**:540–547.

Zohn IE, Sarkar AA. The visceral yolk sac endoderm provides for absorption of nutrients to the embryo during neurulation. *Birth Defects Res A Clin Mol Teratol* 2010;**88**:593–600.