Review: Alterations in placental glycogen deposition in complicated pregnancies: Current preclinical and clinical evidence

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Abstract

Normal placental function is essential for optimal fetal growth. Transport of glucose from mother to fetus is critical for fetal nutrient demands and can be stored in the placenta as glycogen. However, the function of this glycogen deposition remains a matter of debate: It could be a source of fuel for the placenta itself or a storage reservoir for later use by the fetus in times of need. While the significance of placental glycogen remains elusive, mounting evidence indicates that altered glycogen metabolism and/or deposition accompanies many pregnancy complications that adversely affect fetal development. This review will summarise histological, biochemical and molecular evidence that glycogen accumulates in a) placentas from a variety of experimental rodent models of perturbed pregnancy, including maternal alcohol exposure, glucocorticoid exposure, dietary deficiencies and hypoxia and b) placentas from human pregnancies with complications including preeclampsia, gestational diabetes mellitus and intrauterine growth restriction (IUGR). These pregnancies typically result in altered fetal growth, developmental abnormalities and/or disease outcomes in offspring. Collectively, this evidence suggests that changes in placental glycogen deposition is a common feature of pregnancy complications, particularly those associated with altered fetal growth.

Keywords: placental glycogen, diabetes, IUGR, preeclampsia, rodent models, glycogen trophoblast cells
Introduction

Delivery of a healthy baby of optimal weight and size is largely dependent upon a well-functioning placenta. The placenta functions as the interface between the maternal blood supply and the developing fetus and is responsible for the oxygen and nutrient delivery required for normal fetal growth. Many perinatal complications including miscarriage, abnormal fetal growth and even some fetal defects can be attributed to poor placental function [1, 2]. In addition, major obstetric complications such as early onset preeclampsia [3] also directly involve placental dysfunction. However, the impact of poor placental function does not cease upon delivery. There has been renewed interest in placental biology due to our understanding that many diseases of adulthood, including cardiovascular disease, diabetes and even mental disorders, have their origins in utero [4].

One aspect of placental morphology that appears to be indicative of alterations to normal fetal growth is excessive glycogen deposition. This review will begin by briefly summarising the importance of the placenta for short and long-term health outcomes in offspring and the pregnancy complications that can interfere with this role. It will then present current knowledge on the normal process of glycogen storage and handling in the placenta during pregnancy, in both human and preclinical rodent models, and how this is thought to link to fetal nutrient demands and growth. Finally, preclinical and clinical evidence for altered glycogen deposition during various pregnancy complications will be summarised and discussed.

The impact of pregnancy complications on placental function, fetal growth and long-term offspring health
The placenta regulates the extent to which a healthy birth weight is achieved. Glucose and amino acids are the primary energy substrates required by the developing fetus and placenta and reductions in their availability result in reduced fetal growth and low birth weight [5]. The health and nutritional status of the mother can directly influence the availability of these substrates and thus impact on placental development [6]. This impairment often manifests as poor trophoblast invasion and spiral artery remodelling, or reduced vascularization and expansion of the villous tree. This can cause poor placental perfusion or a reduced surface area for substrate exchange respectively [7]. These disruptions can reduce the amount of glucose or amino acids transferred to the fetus and cause growth restriction.

In the human, many obstetric complications are directly related to placental abnormalities. The best characterized is preeclampsia (PE) which affects ~5-10% of pregnancies worldwide and is a major cause of preterm delivery and perinatal morbidity [8]. PE, particularly early-onset PE, significantly increases the risk for fetal growth restriction and this is thought to be due to complications in spiral artery remodelling leading to placental insufficiency [9]. Of great interest in current society is the effect of excess maternal glucose and nutrients which occur in maternal diabetes or obesity. With more than 40% of pregnant women being overweight or obese [10], there are increasing numbers of women with type 2 diabetes entering pregnancy or subsequently developing gestational diabetes. This often contributes to the delivery of large for gestational age (LGA) babies [11]. In diabetic pregnancies, the placenta undergoes structural and functional changes dependent on the modality and quality of glycemic control [12]. These include alterations to the insulin/IGF system [13] as well as cytokines similar to those found in adipose tissue that regulate insulin action [12]. Additionally, factors regulating glucose and lipid metabolism such as FGF21 and GLUT3 are dysregulated in diabetic placentas [14].
Rodent models also provide evidence that pregnancy complications can affect placental function and fetal growth. Maternal hypoxia, which in humans may be caused by smoking, asthma or sleep apnea, has been shown to alter placental development and expression of glucose transporters in mouse models [15, 16]. This limits both oxygen and nutrients to the fetus, resulting in intrauterine growth restriction (IUGR).

Early studies associating fetal growth and adult health outcomes found the most severely affected individuals were those with a low birth weight to placental weight ratio. These individuals had a blood pressure ~25 mmHg higher than the groups with the healthiest birth and placental weights [1]. Other studies suggested a U-shaped relationship between the birth weight to placental weight ratio, as males born with placentas large or small for their body weight have an increased incidence of coronary heart disease as adults [17]. This dichotomy may be due to limited nutrient transfer in a small placenta versus diversion of maternal nutrients by a large placenta to meet its own needs. Collectively, these studies indicate the importance of a normally functioning placenta capable of balancing maternal-fetal nutrient exchange to fetal outcomes and long-term offspring health.

**Glycogen deposition in the human placenta during pregnancy**

Glycogen, a form of stored glucose, is readily deposited in tissues where glucose needs to be easily mobilised such as the liver and skeletal muscle. Whilst it is known the placenta can also store glycogen, there are relatively few studies examining glycogen in the human placenta.

In normal pregnancies, glycogen accumulation begins as early as the first trimester [18], but declines towards term [19]. A region in the human placenta known as the basal plate (bp) contains multilayered columns of cytotrophoblast cells, located at the ends of villi, and extravillous cytotrophoblast cells (EVT). EVT proximal to the villi contain low amounts of
glycogen while the distal EVT are vacuolated and glycogen rich [20]. Strong staining for glycogen has also been reported in cytotrophoblast cells of chorionic villi in the placenta in early pregnancy, gradually decreasing in intensity as the pregnancy progresses [21]. In late first trimester placenta, some fibroblast cells also contain glycogen aggregates [22], but it is not clear if this continues throughout gestation or if it is affected by pregnancy complications. Transport of glucose from mother to fetus is particularly critical to meet fetal nutrient demands and is controlled by a number of specific placental glucose transporters (see [23] for review). In the placenta, glucose can be converted into glycogen for storage either via the classical pathway (glycogen synthase) or through using glycogenin as a primer [18]. In healthy human placenta, it appears that glycogen synthesis via the classical pathway and degradation occurs in syncytiotrophoblasts, cytotrophoblasts and the decidua in the first trimester of pregnancy but is absent at term [24]. In term placenta, glycogen synthesis via the glycogenin pathway is stronger in endothelial cells, syncytiotrophoblasts, extravillous trophoblasts and basal decidual cells than in first trimester [18]. In the decidua, glycoprotein production helps to provide nutrients early in gestation and glycogen synthesis and breakdown may contribute by providing substrates to this process. The metabolically active Hofbauer cells express low levels of glycogen synthase but their expression of glycogen phosphorylase is more pronounced both early in gestation as well as at term [22]. However, glycogen aggregation does not occur in Hofbauer cells even though the expression of GLUT3 suggests that glucose is transported into these cells [22]. This suggests that Hofbauer cells, which are placental macrophages, are not a site for glycogen storage in healthy human placentas at term.

**Glycogen deposition in the rodent placenta during pregnancy**
In the mouse, more is known about glycogen levels in the placenta during pregnancy and in particular, the development of specialised glycogen trophoblast cells (GlyT) cells. GlyT are born in the centre of the ectoplacental cone (EPC), an earlier structure that gives rise to the junctional zone (jz) around E7.5. The jz is centrally located within the mouse placenta and is thought to provide physical support to the underlying vascular exchange structures [25], as well as serving an important endocrine role [26, 27]. GlyT cells share a common lineage with the endovascular invading, spiral artery remodelling trophoblast giant cells [28]. From ~E10.5-E12.5, GlyT begin to accumulate glycogen and can be found as tightly packed clusters embedded within spongiotrophoblast cells (SpT) (Fig. 1). After E12.5, GlyT begin to break out of the jz and invade interstitially into the maternal decidua [29] and congregate around spiral arteries and former sites of uterine natural killer cells (uNK) [30]. Interstitial invasion of GlyT continues until E18.5, at which time a 50% decline in numbers is observed [31]. Glycogen stores have also been reported to decline in the jz of the rat placenta between E16 and E19 by more than 3-fold [32]. Interestingly, GlyT then enter a lytic/apoptotic phase near the end of gestation (~E18.5), forming large glycogen filled lacunae in the vicinity of vascular sinuses, suggesting they provide a source of energy at the end of term or perhaps influence parturition [28].

Superficially, the human and mouse placenta are quite different, although considerable regional and cellular analogies exist [20]. As discussed above, glycogen deposition occurs in more placental cell types within the human placenta than in rodents, which have glycogen specifically in GlyT cells. The jz of the rodent placenta is thought to be analogous to the human bp. In addition, the interstitial invasion by GlyT cells into the maternal decidua late in gestation is comparable to interstitial EVTs that invade deep into the decidua of the human placenta. The low glycogen EVTs proximal to the villi are reminiscent of murine SpT cells, while the high glycogen distal EVTs are similar to murine GlyT cells [20]. Also, many of the
genes known to regulate placental formation in the mouse are expressed in analogous cell types in human placenta [25] and there are many shared genetic pathways utilized in placentation by both species [20]. Importantly, the compartmentalization of glycogen storage to a specialized glycogen trophoblast subtype within the mouse placenta offers a unique opportunity to manipulate putative genes involved in placental glycogen handling and interrogate the effects of such manipulations on fetal growth and development.

What is the putative function of glycogen in the placenta?

As discussed above, glucose can be stored in the placenta in the form of glycogen in both human and rodent pregnancies. However, the function of this glycogen deposition remains a matter of debate and, certainly, its role in complicated pregnancies that result in changes to fetal growth are unknown. At least in healthy pregnancies, stored glycogen is hypothesized to be important for times when feto-placental demand for glucose exceeds supply from the maternal circulation, such as late in gestation [31]. Constitutive placental glycogen storage appears to vary during pregnancy in a sex-dependent manner, at least in the spiny mouse where this has been examined in some detail, with female fetuses showing higher levels of placental glycogen, peaking around mid-gestation [33]. In a poorly functioning placenta such as in IUGR pregnancies, placental glycogen accumulation may reflect storage for its own use, or inability to liberate glucose from the glycogen stores when needed. This would in theory result in reduced transport of glucose to the fetus and thus could be causal in the development of fetal growth restriction. An alternative hypothesis, specifically in cases of a high maternal glucose environment (e.g. diabetes), is that the placenta mounts a compensatory response and stores glycogen to limit excess glucose reaching the baby. Understanding placental glycogen biology, and the circumstances under which it is stored and/or released, may provide insights into pregnancies resulting in inappropriate fetal growth.
Detection of glycogen in placental tissues

There are various methods that have been used for detection of glycogen in human and rodent placental tissue. For histological examination of glycogen deposits in tissue sections, often a simple Haematoxylin and Eosin (H&E) stain is used (for examples see [34-38] in Table 1 and Fig. 1), although this doesn’t specifically stain glycogen. Periodic Acid Schiff (PAS) is the primary stain used as an indicator of glycogen in the placenta (for examples see [33, 39-43] in Table 1 and Fig. 1), although it is not strictly glycogen-specific as it also stains glycoproteins, glycolipids and mucins in addition to the polysaccharides of glycogen. Therefore, use of PAS in combination with amylase or diastase, both of which break down glycogen, can be used to compare against PAS staining alone to confirm glycogen deposits.

Best Carmine’s stain, an ammoniated carminic acid, specifically stains glycogen and has been used to stain mouse placenta [44] (Fig. 1). These stains allow the specific cells and location of glycogen accumulation to be identified in the placenta, but their quality is affected by both the fixative and temperature of fixation [45] and they provide more of a descriptive rather than a quantitative measure of glycogen. Nevertheless, stereological assessment of placental morphology is seen as an important technique for comparing normal pregnancies and those complicated by conditions such as preeclampsia/IUGR and asthma [46].

Recently, a histochemical staining method has been developed that utilises biotinylated BSA-II (the lectin from Bandeiraea simplicifolia-II) which shows binding affinity for intracellular amylase-sensitive glycogen [21]. This method strongly stains cytotrophoblast cells in chorionic and anchoring villi in the placenta in early pregnancy. Ongoing differentiation of these cells during pregnancy results in a gradual decrease in staining intensity, suggesting that this lectin could be a useful marker for these cells in perturbed pregnancies where this differentiation may be affected.
Alternatively, many studies quantify glycogen content by homogenizing the tissue, extracting the glycogen [47, 48] and then using biochemical assays to calculate glycogen content, often relative to total placental protein (as per [49]). Others have utilised assays of glycogen synthase or glycogen phosphorylase activity (as per [48]) as measures of glycogen metabolism. However, although quantitative, these assays do not indicate which placental cell types are involved in glycogen storage.

These different methods for assessing glycogen can result in conflicting conclusions with respect to alterations to placental glycogen under pathological scenarios. Indeed, although there are many examples in which glycogen levels have been shown to be altered (Table 1), some studies have shown no link between placental glycogen and pregnancy complications (for example [50-53]). Different methodologies may be a factor, but these studies also typically have small sample sizes, especially those conducted using human tissues. Additionally, male and female placentas are rarely investigated separately, and few studies investigate glycogen rich EVTs within the basal plate; the cells most analogous to rodent GlyT cells. As a further complication in mouse models, genetic background has been shown to impact on glycogen storage in the placenta, at least in the C57/Bl6 (Bl6) and 129S2/SvHsd (129) strains that have been closely investigated [54]. The Bl6 strain was shown to accumulate 2-fold higher glycogen in late gestation compared to the 129 strain, potentially protecting embryos from IUGR in situations where defects in the jz may affect glycogen storage [54]. Collectively, these methodological factors can produce variability in comparisons between disease states and normal pregnancies.

**Preclinical evidence of altered placental glycogen in rodent models of perturbed pregnancies**
In animal studies, there are a large number and range of maternal conditions and/or environmental exposures that alter placental GlyT and glycogen stores. For example, exposure to the cancer drug cisplatin decreases GlyT numbers and alters the migration of these cells [55]. Moreover, a wide range of programming and disease models result in alterations in glycogen deposition within the junctional zone (Table 1). These alterations occur for various insults across different species and pregnancy time-points, all of which result in altered fetal growth, developmental abnormalities and/or disease outcomes in offspring. Alterations in placental GlyT are more profound in some models but occur in all the models to some degree. Interestingly, changes in placental GlyT are often sex-specific (Table 1), reflecting the sex-specific fetal outcomes that are often seen in perturbation models. Given the variety of preclinical models of pregnancy insults that demonstrate changes in placental glycogen, this suggests a potentially causal mechanistic pathway rather than an epiphrenomenon. Table 1 is not designed to be an exhaustive list of all studies in which glycogen has been recorded, but rather to show the breadth of pregnancy perturbation models in which alterations in GlyT and/or glycogen stores have been reported.

Mouse knockout studies have shed some light on the genes responsible for GlyT differentiation [56, 57]. The imprinted gene Phlda2 appears particularly central to GlyT development, as an up-regulation of Phlda2 expression results in a significant decrease in GlyT numbers and placental glycogen content, while decreased Phlda2 expression results in increased GlyT numbers and glycogen content [49, 58]. Remarkably, either loss or excess of GlyT and placental glycogen were associated with fetal growth restriction. While this is strong evidence that dysregulated placental glycogen handling results in inappropriate fetal growth, some caution is required in interpreting these observations; most gene knockouts affect multiple trophoblast lineages, making it difficult to directly link fetal growth abnormalities with aberrant GlyT biology specifically. Furthermore, GlyT cells may have
additional functions during pregnancy. GlyT invade the decidua and congregate near spiral arteries, possibly playing a role in their remodelling by breaking down the smooth muscle wall or extracellular matrix (ECM) to aid in endovascular trophoblast invasion [59]. GlyT secrete prolactin-like hormones (PRL7B1 and PRL2A1 for example) [60] and retinoic acid [44], and thus likely have an endocrine role also. In fact, GlyT become the main source of IGF2 during the last half of gestation [61], and placental-specific loss of IGF2 results in fetal growth restriction [62]. Future studies that manipulate genes specifically in the GlyT population are needed to clarify the relationship between placental glycogen handling and fetal growth.

Furthermore, in all animal exposure models and most gene knockout models studied to-date, altered GlyT morphology and/or numbers have only been investigated in late gestation, making it difficult to interpret the nature or origin of this dysfunction. For example, an increase in the area occupied by placental GlyT at term may result from an earlier increase in GlyT proliferation, beyond the 250-fold increase normally seen throughout gestation [31]. Alternatively, GlyT invasion into the decidua may be impaired, leaving behind more GlyT within the jz than is normally observed at term, or GlyT may have failed to lyse at the end of gestation. Moreover, increased area occupied by GlyT may represent larger GlyT cells rather than more, as is seen in female placentas at term in the Spiny Mouse glucocorticoid exposure model (Table 1, [33]). This would implicate metabolic dysfunction rather than impacts on their developmental origins. Understanding the origin of the defect is critical to understanding how altered GlyT cells may contribute to placental dysfunction and perturbed fetal growth.

Clinical evidence of altered placental glycogen in complicated pregnancies
In human pregnancies, two pathologies have primarily been associated with altered glycogen metabolism and/or deposition: PE, often resulting in IUGR, and diabetes. More than 20 years ago, it was observed in placentas from pregnancies complicated by PE that there was an increase in trophoblast glycogen content and glycogen synthase kinase (GSK) activity compared to placentas from normotensive, gestational age-matched pregnancies [63]. These differences were most pronounced in microvesicles isolated from their syncytiotrophoblasts [63]. The authors suggested glycogen accumulation may be a metabolic marker of immaturity of this cell which is unable to divide. This field received little subsequent investigation but data emerged almost a decade later to further implicate placental glycogen synthesis and deposition in PE [3]. This included evidence for increased expression of glycogen phosphorylase [64, 65], as well as increased activity of glycogen phosphorylase and glycogen synthase [63]. Increased glycogen deposits were also identified in cytotrophoblasts of terminal chorionic villi by ultrastructural imaging [66]. However, not all studies have reported differences in placental glycogen content in PE [52, 53]. As mentioned above, there may be various reasons for this. Nevertheless, other studies suggest that glycogen storage and/or turnover may be altered in PE. Interestingly, whilst diabetes, mostly type 1 diabetes or gestational diabetes mellitus (GDM), reduces maternal glycogen stores in peripheral tissues, the human placenta has increased glycogen stores in diabetic compared to non-diabetic pregnancies [19]. This has been observed as small deposits of glycogen in the interstitium of terminal villi at term [67], subsyncytially and in the basement membrane [39, 68]. Another histochemical study of the human placenta identified that the majority of the glycogen was deposited in cells surrounding the fetoplacental vasculature with little in the cytotrophoblast cells [69]. This suggests that the glucose contributing to the glycogen accumulation derives from the fetal rather than the maternal circulation and the placenta serves as a buffer for this excess fetal
glucose. Thus, fetal macrosamia should only occur in cases when fetal hyperglycaemia exceeds the placenta’s capacity to store excess fetal glucose [69].

There are some tantalizing indications that the mode of treatment of gestational diabetes affects overall placental glycogen content. Women treated with insulin have similar glycogen content to healthy women, whereas women undergoing a diet-intervention have only slightly reduced placental glycogen levels which may be related to the expression of insulin receptors in trophoblasts [70]. Primary trophoblast cells from healthy term deliveries cultured in 10 mM glucose display intracellular glycogen ‘lakes’ independent of lipid concentrations [71], which suggests that hyperglycaemia rapidly increases glycogen deposition in the placenta.

Glycogen deposition in the human placenta has not been studied in detail in most other complications of pregnancy. For example, a study examining placental morphometry in patients with asthma during pregnancy did not investigate glycogen deposition [72]. Several studies that included placentas from IUGR offspring reported no difference in glycogen deposition with PAS staining [39], or with electron microscopy [73]. The protein and phosphorylation levels of GSK were also not different in pregnancies affected by IUGR, despite decreased expression of upstream signalling proteins including IGF-1 receptor and AKT [74]. However, not all enzymes involved in glycogen synthesis have been studied in human placentas resulting in IUGR.

**Conclusions and future areas for research**

Collectively, there is accumulating data to suggest changes in placental glycogen deposition are a hallmark feature of a compromised pregnancy associated with fetal growth restriction and also, paradoxically, with fetal overgrowth. Future studies should use a combination of robust stereological and biochemical techniques to more thoroughly assess glycogen deposition and metabolism in the human placenta in a range of pregnancy complications.
Additionally, researchers using preclinical models of fetal growth restriction should examine placental glycogen and should importantly, determine if differences are sex-specific. Studies in preclinical rodent models also allow investigation of the placenta across gestation enabling the potential temporal changes of placental glycogen to be elucidated.

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References


[38] K. Bibeau, B. Sicotte, M. Beland, M. Bhat, L. Gaboury, R. Couture, J. St-Louis, M. Brochu, Placental Underperfusion in a Rat Model of Intrauterine Growth Restriction Induced by a Reduced Plasma Volume Expansion, PloS one 11(1) (2016) e0145982.


Table 1: Preclinical models demonstrating altered placental glycogen trophoblast cells (GlyT) in perturbed pregnancies.

<table>
<thead>
<tr>
<th>Maternal Insult</th>
<th>Species</th>
<th>Exposure/Treatment</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Rat^</td>
<td>Periconceptional (E-4 to E4); 12.4% v/v EtOH</td>
<td>↑ GlyT cell area in female term placentas (Fig. 1); IUGR</td>
<td>[34]</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Rat</td>
<td>Early to mid-gestation (E6.5 to E13.5); 11% O₂</td>
<td>↑ endovascular GlyT invasion when hypoxia exposure between E6.5-E10.5; ↑ JZ/LZ ratio at E13.5</td>
<td>[75]</td>
</tr>
<tr>
<td>Hypomagnesemia</td>
<td>Mouse^</td>
<td>4 weeks prior and throughout gestation; 0.02% and 0.005% w/w Mg²⁺</td>
<td>↑ GlyT cell area in male and female placentas; IUGR and ↑ postnatal loss</td>
<td>[35]</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>Rat</td>
<td>Throughout gestation; partial thyroidectomy</td>
<td>↑ glycogen content by glucoamylase digest at E19 and at term in junctional zone; IUGR</td>
<td>[32]</td>
</tr>
<tr>
<td>IVF</td>
<td>Mouse</td>
<td>Prior to gestation; comparison of control, IVC and IVF</td>
<td>↑ GlyT cell area in IVC and IVF; no change in Pcdh12 (GlyT marker); ↑ Gys2 (glycogen metabolism) and ↑ Tpbpa (JZ marker); IUGR</td>
<td>[42]</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Rat</td>
<td>Throughout gestation; STZ treatment prior to mating (35-60mg/kg)</td>
<td>↑ GlyT numbers at term by histology; ↑ glycogen content by protein assay from E16; ↑ glycogen synthase/ phophorylase activity</td>
<td>[19, 40, 41, 48]</td>
</tr>
<tr>
<td>Hyperglycaemia</td>
<td>Rat</td>
<td>Late gestation (E18 to E20); 40% glucose infusion</td>
<td>↑ glycogen content by protein assay at E20.5</td>
<td>[76]</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>Spiny mouse^</td>
<td>Mid-gestation (E20.5 to E23); 125µg/kg DEX</td>
<td>↑ GlyT cell area at E37 (term is E39) due to GlyT hyperplasia in males and GlyT hypertrophy in females</td>
<td>[33]</td>
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Table 1: Continued.

<table>
<thead>
<tr>
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<th>Findings</th>
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<tbody>
<tr>
<td>Placental underperfusion</td>
<td>Rat</td>
<td>Late gestation (E16-E22); low sodium diet (0.03%) to reduce plasma volume expansion</td>
<td>↑ GlyT cells in JZ at E22; IUGR</td>
<td>[38]</td>
</tr>
<tr>
<td>Placental underperfusion</td>
<td>Rat</td>
<td>Late gestation (E17); uterine ischemia/reperfusion injury for 30 mins</td>
<td>↓ GlyT cell area (by 50%) at E18 in JZ; IUGR</td>
<td>[36]</td>
</tr>
<tr>
<td>Placental underperfusion</td>
<td>Rat</td>
<td>Mid-late gestation (E10-E20); ligation of abdominal aorta E10</td>
<td>↓ GlyT cells at E15 and E20</td>
<td>[77]</td>
</tr>
<tr>
<td>Protein restriction</td>
<td>Rat</td>
<td>Throughout gestation; low protein diet (6% casein)</td>
<td>↓ GlyT at E17 and E19; ↓ JZ/LZ at E17; IUGR</td>
<td>[37]</td>
</tr>
<tr>
<td>Protein restriction</td>
<td>Mouse</td>
<td>2 weeks prior and throughout gestation; low protein diet (6% casein)</td>
<td>↓ GlyT cells at E10.5 and E17.5 by <em>Pcdh12</em> mRNA; ↓ JZ from E10.5 by <em>Tpbpa</em> ISH (~20-30%); IUGR</td>
<td>[78]</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Rat</td>
<td>Throughout gestation; stroke prone spontaneously hypertensive rat (SHRSP strain)</td>
<td>↓ GlyT cells at E18</td>
<td>[43]</td>
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</table>

E = embryonic day. IUGR = intrauterine growth restriction. STZ = streptozotocin. DEX = dexamethasone. JZ = junctional zone. LZ = labyrinth zone. IVF = *in vitro* fertilisation. IVC = *in vitro* culture. ^ = placental sex determined. ISH = *in situ* hybridisation. mRNA = messenger RNA. *Pcdh12* = protocadherin 12; *Tpbpa* = trophoblast-specific protein alpha; *Gly2* = glycogen synthase 2.
Figure Legend

Fig. 1. Glycogen trophoblast (GlyT) cell localization in the rodent placenta. A and B: Haematoxylin and eosin (H&E) staining of embryonic day 20 placentas from untreated/control (A) and periconceptional ethanol (B) exposed rat pregnancies. Main images at 1X magnification (scale bars 1000 µm) and inset images at 20X magnification (scale bars 100 µm). Glycogen stores appear white in section. Images adapted from [34]. C and D: Embryonic day 14.5 mouse placenta stained with Periodic Acid Schiff (PAS) (C) and the glycogen-specific Best’s Carmine stain (D). The dark staining in the junctional zone corresponds with glycogen trophoblast cells. Note that PAS staining is more broad than Best’s Carmine, likely due to the wider range of substrates detected by PAS such as other glycoproteins, glycolipids and mucins. Scale bars as per A and B.
**Conflict of interest statement:**

The authors have no conflict of interest.