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## TOPICAL REVIEW

# Guinea pig models for translation of the developmental origins of health and disease hypothesis into the clinic

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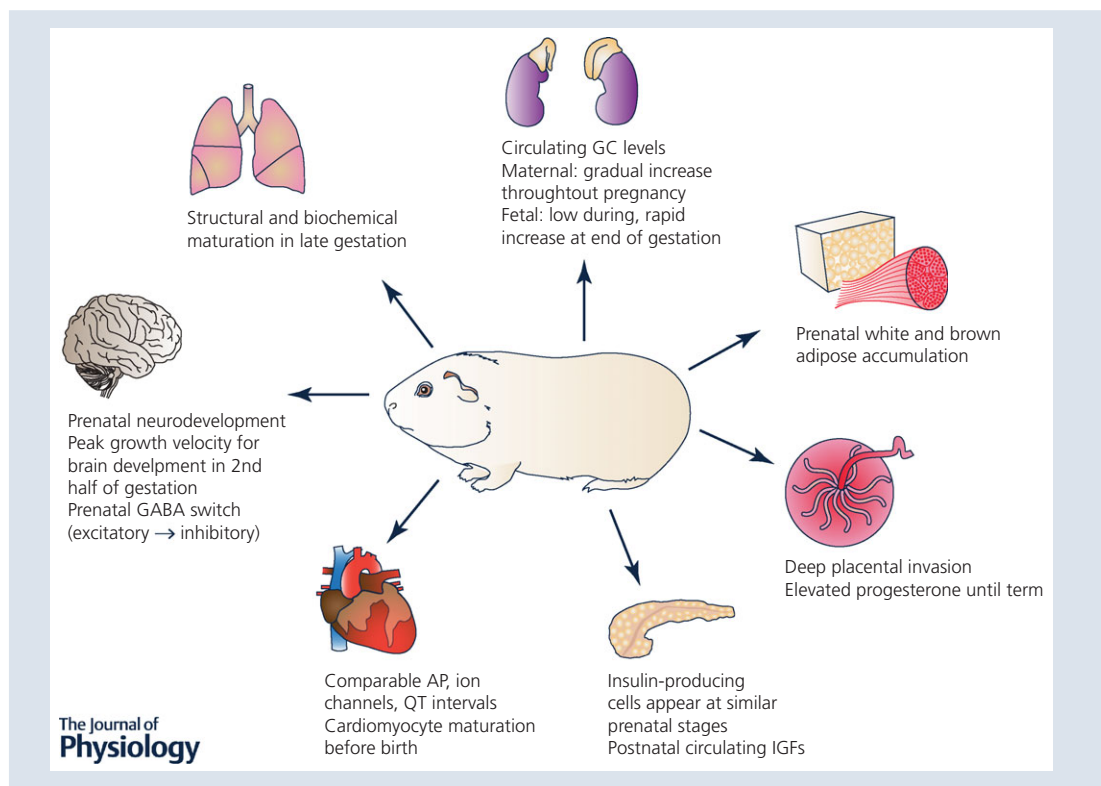
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**Abstract** Over 30 years ago Professor David Barker first proposed the theory that events in early life could explain an individual's risk of non-communicable disease in later life: the developmental origins of health and disease (DOHaD) hypothesis. During the 1990s the validity of the DOHaD hypothesis was extensively tested in a number of human populations and the mechanisms underpinning it characterised in a range of experimental animal models. Over the past decade, researchers have sought to use this mechanistic understanding of DOHaD to develop therapeutic interventions during pregnancy and early life to improve adult health. A variety of animal models have been used to develop and evaluate interventions, each with strengths and limitations. It is becoming apparent that effective translational research requires that the animal paradigm selected mirrors the tempo of human fetal growth and development as closely as possible so that the effect of a perinatal insult and/or therapeutic intervention can be fully assessed. The guinea pig is one such animal model that over the past two decades has demonstrated itself to be a very useful platform for these important reproductive studies. This review highlights similarities in the *in utero* development between humans and guinea pigs, the strengths and limitations of the guinea pig as an experimental model of DOHaD and the guinea pig's potential to enhance clinical therapeutic innovation to improve human health.

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**Abstract figure legend** Similarities in timing of development and responses between guinea pigs and humans.

The authors of this review represent laboratories, including clinicians, scientists, laboratory heads, post-doctoral fellows and trainees, from around the world that study the developmental origins of health and disease (DOHaD). These groups use a range of animal models, one of which is the guinea pig, to understand why and how changes in the fetal and neonatal environment are associated with an increased risk of non-communicable disease in adult life. Together, using guinea pigs as a model of human development, this work can answer important mechanistic questions about DOHaD and aid in the design and testing of interventions aimed at reducing the impact of non-communicable disease on quality and quantity of life around the world.



## Introduction

Originally describing the association between early life nutrition and the increased risk of early onset cardiovascular and metabolic diseases, Barker and others, such as Neel and Forsdahl, through the 1960s and into the 1980s developed and put forward the concept of early life reprogramming (Neel, 1962) and later specifically fetal programming (Forsdahl, 1977). Further to this, large-cohort epidemiological studies not only confirmed but strengthened the argument (Barker, 2004; Gluckman & Hanson, 2004) ultimately leading to Barker's description of the developmental origins of health and disease (DOHaD) hypothesis (Barker *et al.* 1990, 2002; Barker, 2005). Today, it is widely acknowledged that suboptimal *in utero* or early life conditions result in permanent alterations to fetal developmental and growth trajectories due to structural, functional and epigenetic changes and that these are associated with increased metabolic disease risk later in the life cycle.

While epidemiological studies in human populations have provided robust evidence linking suboptimal growth

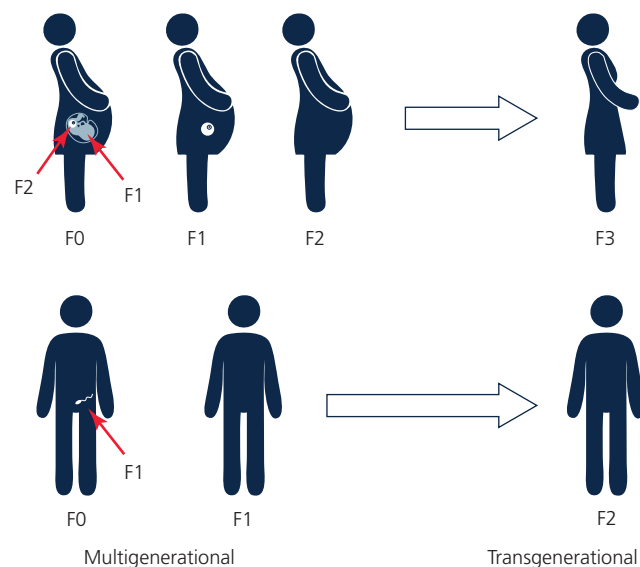
*in utero* with adverse health outcomes in adult life, such associations cannot establish causality nor define the mechanisms through which these adult health consequences are mediated. Instead, the use of appropriate animal models of DOHaD has allowed us to understand some of the pathophysiological and mechanistic basis for the effect of early life perturbation on long-term health and wellbeing (Barker *et al.* 1990, 2002; Barker, 2005; Dickinson *et al.* 2016). Additionally, prospective and interventional studies in humans would take decades to determine outcomes in only the F1 generation, further supporting the need for animal model systems with relatively short reproductive timelines. This latency between understanding the mechanistic basis of disease risk through to development of a therapeutic strategy and assessing its potential efficacy is far too long. Thus, animal models play important roles both in determining the mechanisms that link growth *in utero* and early life to health in adult life and in development of novel therapeutic interventions.

Although a range of species (sheep, rats, mice, spiny mouse) have been used to investigate DOHaD, in this

review we will outline the strengths of using pregnant guinea pigs (*Cavia porcellus*), as well as potential limitations, as an experimental model for the study of DOHaD. We present how studies in this species have contributed and continue to contribute to our understanding of DOHaD, generating data to lead clinical innovation in this area.

### DOHaD has multigenerational and transgenerational effects

Notably, the consequences of perinatal exposures are not restricted to the offspring in the F1 generation but also occur in the F2 and F3 generations (Fig. 1). For example, grand-paternal food supply is associated with a grandson's mortality risk ratio whilst grand-maternal food supply is associated with a granddaughter's mortality risk ratio (Pembrey *et al.* 2006). These persistent multigenerational effects reflect exposures of the granddaughter's oocyte, present within her mother when her mother was a fetus in her grandmother (Dickinson *et al.* 2016). Changes in metabolism in the F1 generation may also mediate some effects on subsequent generations, whilst epigenetic changes are proposed to underlie some persistent effects of early life exposures that are transgenerational as well



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#### Figure 1. DOHaD studies have shown that exposures in one generation can have multigenerational and intergenerational effects

Changes in the grandmother (F0) or grandfather (F0) can influence the health of the granddaughter (F2) or son (F1), respectively. Transgenerational effects have also been identified that affect the great granddaughter (F3) and the grandson (F2). Adapted from Dickinson *et al.* (2016) with permission.

as multigenerational (Fig. 1), including those transmitted through the male line (Burdge *et al.* 2007; McPherson *et al.* 2015).

### Mechanistic DOHaD studies require a clinically relevant animal model

The need for translational animal models of high-risk pregnancy and adverse pregnancy outcomes remains a research priority, in part due to the relative paucity and ethical concerns of interventional human studies in this population. Understanding the mechanistic basis of multi- and transgenerational developmental programming requires studies of DOHaD in a small animal species with a relatively short lifespan compared with humans. Although large animal species such as the sheep are also widely used in DOHaD studies, sheep take longer to reach maturity and have additional housing and logistical requirements compared with small animal species.

For meaningful clinical translation, these animal models must satisfy several conditions. Firstly, that the *in utero* environment is comparable to humans. Secondly that fetal developmental timing is broadly similar to humans, so that *in utero* exposures occur at similar developmental stages to those of the human. As such, the translatability of studies performed in altricial species (mice and rats) should be approached with caution as many key stages of development that occur before birth in humans occur after birth in these species (Dobbing & Sands, 1970; Hunter *et al.* 2016). Thirdly, fetal number should not be excessive. Unlike humans, guinea pigs generally have more than one offspring, but their litter sizes of 1–5 are substantially lower than the 8–12 pups usually seen in rats or mice. Finally, it is crucial that animal models of DOHaD share similar patterns of adverse outcomes to those seen in human studies following the same exposure, so that any therapeutic intervention can be thoroughly evaluated (Shaw *et al.* 2016).

### Fetal environment and developmental timing: similarities between guinea pig and humans

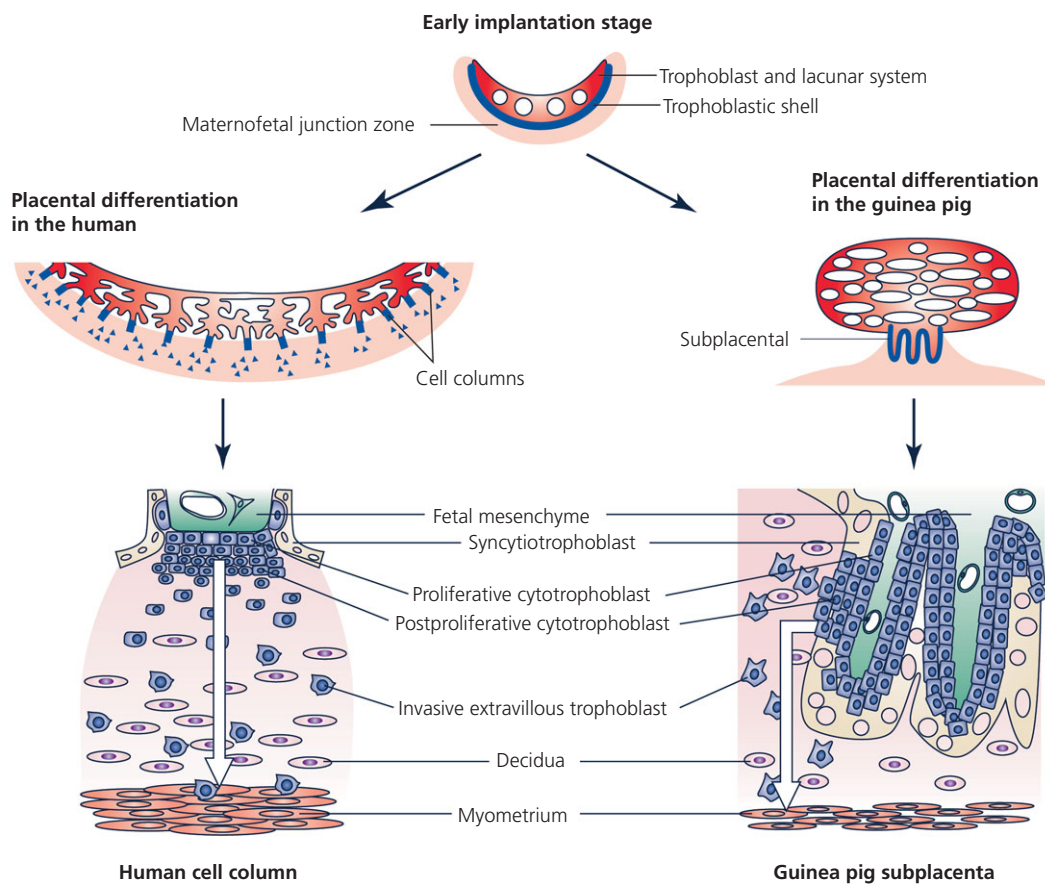
The guinea pig has a relatively long gestation (69–71 days) compared to other rodents such as the mouse and rat (19–21 days), yet shorter than that of the sheep (145–150 days). This longer gestation allows for better temporal resolution of developmental plasticity and identification of critical time periods for vulnerability to insults. Importantly, the timing of development of major organ systems that are impacted by *in utero* exposures or perinatal complications with adverse health outcomes in humans are similar in the guinea pig (Briscoe *et al.* 2004).

## Placenta development

**Placenta morphology.** The guinea pig has a haemomonochorial placenta that is an excellent surrogate for the haemochorial human placenta (Fig. 2; Smith *et al.* 1999; Carter *et al.* 2006; Mess, 2007; Mitchell & Taggart, 2009). Importantly, the guinea pig placenta invades deeply into the decidua similar to the human, in contrast to the shallower invasion of other rodents (Kaufmann *et al.* 2003). The subplacenta is analogous to the cell columns of the anchoring chorionic villi of the human placenta (Carter *et al.* 2006; Mess, 2007) and contains proliferating trophoblast cells of similar subtypes to those of the human (Kaufmann *et al.* 2003; Carter *et al.* 2006) that differentiate and contribute to decidualisation of maternal endometrium and invasion of maternal blood vessels (Kaufmann *et al.* 2003; Mess, 2007). The labyrinthine region of the guinea pig placenta contains distinct units of maternal and fetal blood vessels separated by a fetal syncytiotrophoblast layer and functions to exchange oxygen/nutrient supply between these two circulations (Fig. 2). However, the guinea pig placenta functions as

a countercurrent gas exchanger (Miglino *et al.* 2004), whereas the human placenta appears to function as a concurrent gas exchanger (Wilkening & Meschia, 1992; Lin *et al.* 2016).

**Placental and reproductive endocrine function.** Like humans, pregnant guinea pigs exhibit a luteo-placental shift in hormone production so that the placenta acts as the major progesterone source throughout the major part of pregnancy (Heap & Deanesly, 1966). Similar to pregnancy in humans and non-human primates, circulating progesterone remains elevated until term (Challis *et al.* 1971; Illingworth *et al.* 1971; Mitchell & Taggart, 2009). This contrasts with most other laboratory species, including mice, rats and sheep (Fig. 3A), which are dependent on systemic progesterone withdrawal to initiate parturition (Mitchell & Taggart, 2009). Placental progesterone production in guinea pigs means that, similar to the human, maintenance of pregnancy is independent of the ovary in the second half of pregnancy (Csapo *et al.* 1981). Conversely, ovariectomy induces



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**Figure 2. Structural similarities between guinea pig and human placenta**

Reproduced with permission from Mess (2007).



parturition and/or pregnancy loss in mice and rats throughout pregnancy (Fig. 3B).

Human and guinea pig placentas share similarities in their response to glucocorticoids, allowing effects of endogenous and exogenous glucocorticoids on placental function and fetal programming to be investigated. Similar to the human placenta, the guinea pig placenta expresses multiple isoforms of the glucocorticoid receptor (Saif *et al.* 2016), and metabolises maternal glucocorticoids via  $11\beta$ -hydroxysteroid dehydrogenase type 2, with activity of this enzyme decreasing towards term, resulting in increased fetal exposure to endogenous glucocorticoids (Sampath-Kumar *et al.* 1998; Murphy & Clifton, 2003). It also expresses P-glycoprotein (Kalabis *et al.* 2009), which contributes to the placental glucocorticoid barrier via transport of glucocorticoids back into the maternal circulation (Soo *et al.* 2012; Hodyl *et al.* 2013; Bloise *et al.* 2016).

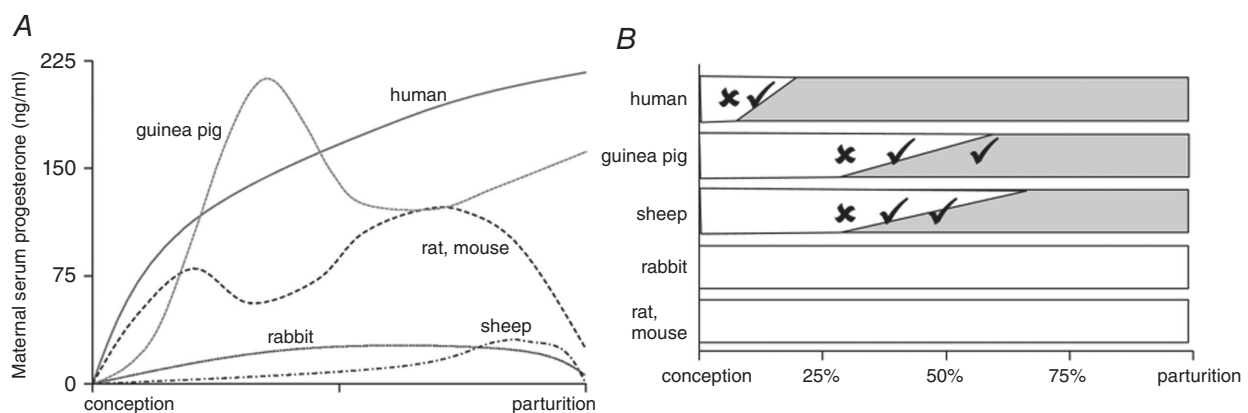
**Placental metabolic function.** The placenta of many species, including guinea pig, is incapable of gluconeogenesis (Bossi & Greenberg, 1972) and any newly synthesized glucose must be of either fetal or maternal origin (Dwyer & Stickland, 1994). Gluconeogenic enzymes are present in the guinea pig liver from gestational day (GD) 40. Similar to sheep and humans (Sadava *et al.* 1992; Houin *et al.* 2015), fetal gluconeogenesis does not take place until late gestation (Jones & Ashton, 1976), meaning the fetus is dependent on glucose delivery from the mother down a concentration gradient. As with glucose, amino acids cross the guinea pig placenta (Jansson & Persson, 1990) and essential and non-essential amino acids and fatty acids can be transferred from the maternal to the fetal circulation against the feto-maternal gradient (Thomas

& Lowy, 1983). These observations are supportive of active amino acid transport mechanisms being present (Jones & Rolph, 1985), similar to human pregnancies. In guinea pigs, like humans but unlike other rodents, maternal circulating concentrations of insulin-like growth factor 1 and 2 are substantial, increasing during pregnancy (Sohlstrom *et al.* 1998), and may have major influences on nutrient partitioning during pregnancy (Sferruzzi-Perri *et al.* 2006).

**Uteroplacental circulation.** Radioactive microsphere studies have demonstrated a correlation between placental blood flow and fetal size even after correction for placental weight differences (Myers *et al.* 1982). This mirrors the human singleton pregnancy where total uterine artery blood volume flow in the first or early second trimesters is positively correlated to birth weight (McKelvey *et al.* 2017), and total uterine blood flow is reduced in fetal growth restricted (FGR) pregnancies (Konje *et al.* 2003). In contrast to humans, lower placental and fetal weights are found naturally in the middle pregnancy sites compared to either the tubal or cervical zones in guinea pigs and other rodents (Turner & Trudinger, 2000), reflecting preferential perfusion of the tubal and cervical uterine zones.

### Fetal development

**Metabolic tissues.** Significant accumulation of adipose tissue in late gestation occurs in fetal guinea pigs and humans. Like the human fetus, but unlike most other species, the guinea pig fetus lays down both brown and white adipose tissue *in utero* and has a total fat content of ~14% at birth (Mace *et al.* 2006), significantly higher than



**Figure 3. Circulating progesterone remains elevated throughout pregnancy in guinea pigs and humans** Circulating progesterone (A) and corpus luteum (CL)-dependence of pregnancy maintenance (B) reflect species differences in placental progesterone production throughout pregnancy. Bars indicate main source of circulating progesterone (white: CL, shaded: placenta). Ticks indicate that pregnancy can be maintained after removal of the ovary or CL, crosses indicate that either the CL or exogenous progesterone is required to maintain pregnancy. A is reproduced from Mitchell & Taggart (2009 with permission). Data in B are from Csapo & Pulkkinen (1978), Csapo *et al.* (1981), Al-Gubory *et al.* (2000) and Bazer (2015).

other rodents and the fetal sheep, but similar (~10%) to the human (Carberry *et al.* 2010). Adipogenesis and fat cell hyperplasia are upregulated during early life but very low in the adult guinea pig (Castaneda-Gutierrez *et al.* 2011). Similarly, in humans, adipocyte number is set before puberty with limited ability to form new adipocytes in adulthood (Spalding *et al.* 2008). Furthermore, the guinea pig develops large epicardial adipose depots (Rolph *et al.* 1982), making them a better model for studies of human cardiac–epicardial depot interactions than rats (Swifka *et al.* 2008).

Timing of myogenesis in the guinea pig also resembles that of humans and species such as sheep (Romero *et al.* 2013) with secondary myogenesis occurring around mid-gestation, in contrast to rats where this occurs later in gestation. In the guinea pig, primary myotubes are observed at GD 30, secondary myotubes appear between GD30 and 35 and fibre hyperplasia is complete by GD50 (Dwyer *et al.* 1995).

Pancreatic development also occurs at similar prenatal stages in guinea pigs as in humans, with insulin-producing cells apparent at ~25% of gestation in human (Reddy & Elliott, 1988) and by ~40% of gestation in guinea pig (Reddy *et al.* 1992). Drug transporters are present in the guinea pig liver in late gestation (Soo *et al.* 2012). Despite these similarities in developmental timing of metabolic tissues, the guinea pig is born relatively more metabolically mature than most other mammals, including humans, since, in addition to suckling milk, it is able to eat solid food from birth (Davis *et al.* 1979).

The timing for ovarian follicle development in guinea pigs is likewise more similar to that in humans than to other rodents. While primordial follicle assembly occurs prenatally between GD48 and 56 in guinea pigs (Bookhout, 1946) and beginning at gestational week 13 in humans (Forabosco & Sforza, 2007), this process occurs during the early post-natal period in other rodents (Bookhout, 1946; Rajah *et al.* 1992; Forabosco & Sforza, 2007).

**Cardiovascular system.** There remain gaps in our knowledge regarding the developmental timing of the fetal guinea pig heart and its comparison to human heart development. The literature does, however, identify a mature fetal heart phenotype in guinea pigs in contrast to other rodent species and in some ways similar to the human fetus. In the fetal guinea pig heart, there is a linear increase in the fraction of cell volume occupied by mitochondria and myofibrils, and a decrease in the fractional volume of nuclei and sarcoplasmic reticulum over the last third of gestation (Rolph *et al.* 1982). These developmental changes are associated with an increase in the number of mitochondria and myofibrils and a decrease in nuclear number and sarcoplasmic reticulum volume, respectively. Increasing contractile

properties in the developing heart are associated with increased organization of cardiac ultrastructure along with increased oxidative capacity of mitochondria (Schaper *et al.* 1985), maturation of sarcomere structure and myofibril organization (Racca *et al.* 2016).

The ultrastructure of the fetal guinea pig heart (Rolph *et al.* 1982) is consistent with the density, elongation and alignment of myofibrils measured in midterm (127 days' gestation) fetal human hearts (Racca *et al.* 2016). The fetal guinea pig heart is more mature at birth in regards to compartmentation of metabolic pathways and organization of mitochondrial ultrastructure than fetal hearts of rat or mouse (Barrie & Harris, 1977; Hoerter *et al.* 1994; Hew & Keller, 2003). Further, the fetal guinea pig heart exhibits a greater reliance on transsarcolemmal calcium (Hew & Keller, 2003), cellular compartmentation of creatine kinase (Hoerter *et al.* 1994) and advanced development of the sarcoplasmic reticulum toward term (Agata *et al.* 1994) providing more mature mechanisms for cardiac contraction. Lastly, the fetal guinea pig has increasing CaATPase activity associated with mitochondria and sarcoplasmic reticulum in late gestation (Rolph *et al.* 1982) and both sympathetic and parasympathetic innervation at the time of birth (De Champlain *et al.* 1970; Friedman, 1972; Hew & Keller, 2003) also identifying a mature cardiac phenotype that differs from other rodent species and is similar to humans. The guinea pig has been used to study functional properties of the heart because action potential configuration, ion channels and the QT interval characteristics are similar to those of the human heart and demonstrate comparable maturational changes during gestation and after birth (Shiotani *et al.* 2008; Nerbonne, 2016). For example, while similarities in K<sup>+</sup> channel characteristics exist among animal species such as guinea pig, human, rat and rabbit (Nerbonne, 2016), developmental changes are complete in the late gestation fetal human and guinea pig heart, whereas this occurs postnatally in rats and mice (Agata *et al.* 1993, 1994).

**Placenta–heart axis.** Fetal heart development is influenced by haemodynamics of the feto-placental circulation, since the fetal heart must eject a blood volume against a systemic impedance determined by downstream vascular beds, including the placenta (Thornburg *et al.* 2010). Thus, disorders of placental development may have effects on fetal cardiac function either directly or indirectly via altered haemodynamic and/or metabolic influences. As both guinea pig fetal heart and guinea pig placenta mirror many of the key phenotypic characteristics of the human fetal heart and placenta (Carter, 2007; Mess *et al.* 2007), experimentally induced placental dysfunction in the guinea pig (Turan *et al.* 2017) may contribute to our understanding of the 'programming' of later heart dysfunction in offspring of a high-risk pregnancy.



**Brain and nervous system.** Using the timing for peaks in brain growth velocity as a marker of development, guinea pigs and sheep can be classified as prenatal brain developers, humans as perinatal brain developers, and other rodents as postnatal brain developers (McIntosh *et al.* 1979). The most rapid phase of synapse formation and myelination is initiated in the latter half of pregnancy in humans and guinea pigs, whilst predominantly occurring in the weeks after birth in other rodents (Dobbing & Sands, 1970; Nitsos & Rees, 1990; Nacher *et al.* 2000; Back *et al.* 2001). The anatomical development of the brain also correlates with its electrophysiological development as indicated by behavioural state maturation. Whereas guinea pigs, sheep and humans have well differentiated and relatively mature electrocortical patterns at birth, rats have a poorly differentiated electrocorticogram at birth (Szeto & Hinman, 1985; Szeto *et al.* 1985; Umans *et al.* 1985). This neurodevelopmental correlation, along with the high proportion of rapid eye movement-like behavioural activity during early life, supports a role for behavioural state activity in the brain's development and more so in guinea pigs and humans prenatally in response to conditions during pregnancy than in rats (Richardson *et al.* 2014).

The immature brain exerts excitatory  $\gamma$ -aminobutyric acid (GABA) activity that is involved in many processes of neurogenesis including neuronal proliferation, migration, differentiation, and oligodendrocyte and synaptic development. A transition from immature (excitatory) to mature (inhibitory) GABAergic activity occurs before birth in humans and primates (Khazipov *et al.* 2001; Sedmak *et al.* 2016), but postnatally in rodents (Rivera *et al.* 1999). Preliminary studies in guinea pigs indicate this transition occurs in the last third of pregnancy (Coleman *et al.* 2013), further supporting the use of guinea pigs to explore the neurodevelopmental impact of pregnancy complications.

**Lung.** Comparable to humans, lung biochemical and morphological development occurs in late gestation in the guinea pig but after birth in rats (Hunt *et al.* 1991). At GD55 the fetal guinea pig's lungs are immature and 'canalicular' (seen in humans from 14 to 23 weeks of gestation; term, 40 weeks), with thick septae and an airspace to tissue ratio of approximately 1:1 (Fig. 4). Although type II alveolar epithelial cells that produce surfactant protein are present, their density increases linearly with age (Lin & Lechner, 1990). By GD58, the lungs have undergone rapid and significant maturation, representative of the 'saccular' stage of lung development (seen in humans from 24 weeks' gestation, the cusp of human postnatal viability, through to term), characterised by decreased tissue density, marked septal thinning, and as a consequence, a marked increase in potential airspace (~73%). Morphological changes from GD58 through

to term are less significant, with slower thinning and septation giving rise to greater potential airspace (~81%) by term. This similar lung developmental timing allows survival of preterm guinea pigs with support much like that provided to preterm neonates in the neonatal intensive care unit (NICU) (Sosenko & Frank, 1987; Kelly *et al.* 1991b; Berry *et al.* 2015).

**Kidney.** Although the guinea pig renal system is relatively less well characterised than other systems, key aspects of maturation and development appear to be similar to that of the human. For instance, like humans, but unlike other rodent species, nephrogenesis in the fetal guinea pig is completed during fetal life (Welling *et al.* 1989) with a trajectory of renin-angiotensin pathway activation that mirrors that of the human (Raimbach & Thomas, 1990).

**Assessment of fetal size and wellbeing.** As with human pregnancy, fetal number, growth trajectory and haemodynamics can be tracked throughout gestation using non-invasive ultrasound techniques in guinea pigs. Optimal images are obtained with a narrow footprint high frequency ultrasound probe (7–15 Hz). Gestation sacs are visible from ~GD20–25 and the fetal measurements obtained are similar to those used for dating and serial ultrasound assessment of human fetal growth and fetal growth in other rodents, ruminants and rabbits. Crown-rump length can be assessed up to GD35 but after that time the fetus is too long and flexed for accurate measurement (Santos *et al.* 2014; Swanson *et al.* 2017). Biparietal diameter, occipito snout length, head circumference and abdominal circumference can be measured from ~GD25 and used to track changes in fetal size as gestation advances (Fig. 5; Turner & Trudinger, 2000, 2009). Blood flow in the umbilical artery, uterine artery and fetal heart can be measured using Doppler ultrasound (Herrera *et al.* 2016). In this way ultrasound can identify *in vivo* fetal growth and/or haemodynamic acute and longitudinal responses to experimentally induced environmental insults as well as to therapeutic intervention.

We have not found major differences in fetal outcomes in models of hypoxia or stress in terms of stillbirth, spontaneous abortion or preterm birth. For example, repeated maternal stress with a strobe light during early and mid-gestation does not change reproductive parameters (Schopper *et al.* 2011), despite down-regulation of the hypothalamic-pituitary-adrenal (HPA) axis and lower weight gain in treatment females. However, maternal undernutrition severe enough to result in fetal growth restriction can lead to preterm delivery (Elias *et al.* 2016; Nevin *et al.* 2018).

In addition, dams must remove the fetal membranes from around the face of each pup and thus, in large litters, she may not get to the last pup quickly enough. These

postnatal deaths are not stillbirths but are primarily a reflection of litter size and the short inter-pup delivery interval. Furthermore, we have not observed dystocia in dams that have had their first mating by 6–8 months of age.

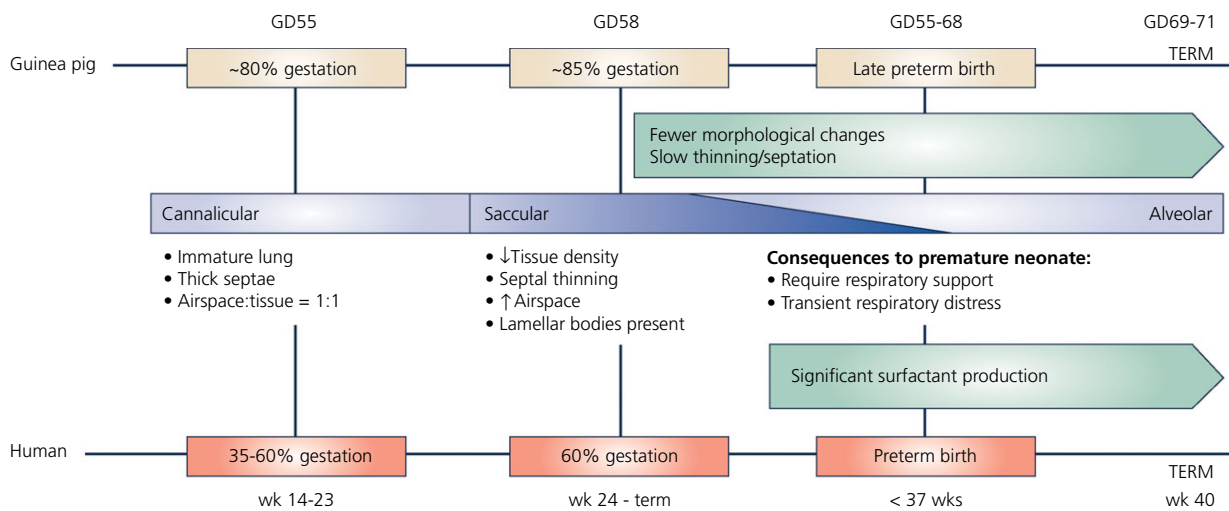
### Models of DOHaD in guinea pigs

The wealth of commonality between guinea pigs and humans in terms of the developmental trajectory of major organ systems illustrates why many researchers choose to work with guinea pigs as a translational model of DOHaD to understand the impact of early life conditions on diverse outcomes including neurodevelopmental, cardiovascular, respiratory, metabolic and reproductive (Table 1). Crucially, as occurs clinically, the pattern of deficit differs depending on the severity, timing

and duration (chronic, acute or repeated) of the perinatal insult (Morrison, 2008). The ensuing functional deficits can be evaluated postnatally using established guinea pig assessment techniques (Bennett *et al.* 2016; Shaw *et al.* 2016). Many guinea pig models of clinically important perinatal perturbation have been established (Table 1) and we have summarised the knowledge that has been gained below. This has positioned the field to now pursue studies of potential therapeutic interventions to improve outcomes.

### Programming of neurodevelopment

The neurodevelopmental consequences of fetal and perinatal adversity (Table 1) may alter total or regional brain structure or volume, cause white matter injury or delay and/or alter neurogenesis. Progesterone, maintained at



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Figure 4. Comparison of the stages of lung development in the human and guinea pig fetus

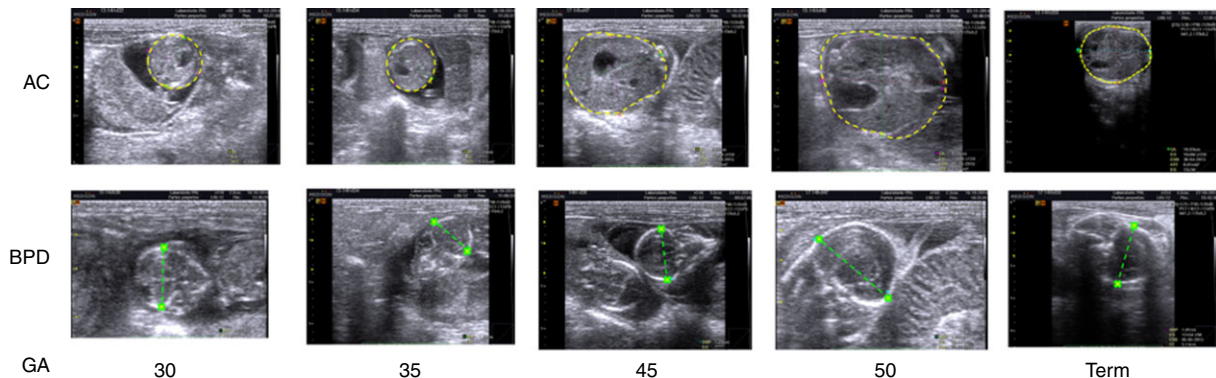


Figure 5. Ultrasound measurement of fetal size throughout gestation in the guinea pig

Images show measurement of abdominal circumference (AC, yellow lines) and biparietal diameter (BPD, green lines) from GD30 until term.

**Table 1. Guinea pig models used in DOHaD research**

Model to induce developmental programming	Specific protocol	Altered fetal or neonatal growth	Organ system investigated (fetal/postnatal time point of analysis)
Chronic maternal hypoxia	10–16% O <sub>2</sub> for varying durations in the second half of pregnancy	Dependent on duration and degree of hypoxic insult, e.g. 12% O <sub>2</sub> for 2nd half of gestation or 10% O <sub>2</sub> ≥ 10 days before term	Heart Fetal (Thompson <i>et al.</i> 2000, 2009; Thompson & Dong, 2005; Dong & Thompson, 2006; Oh <i>et al.</i> 2008 <i>b</i> ; Evans <i>et al.</i> 2012 <i>a,b</i> ; Al-Hasan <i>et al.</i> 2013; Turan <i>et al.</i> 2017) Postnatal (Al-Hasan <i>et al.</i> 2014) Brain Fetal (Guo <i>et al.</i> 2010; Dong <i>et al.</i> 2011; Evans <i>et al.</i> 2012 <i>a,b</i> ; Blutstein <i>et al.</i> 2013) Postnatal (Kim <i>et al.</i> 2015; Wang <i>et al.</i> 2016) Liver, fetal (Oh <i>et al.</i> 2008 <i>a</i> ; Hashimoto <i>et al.</i> 2012; Al-Hasan <i>et al.</i> 2013) Lung, fetal (Oh <i>et al.</i> 2008 <i>a</i> ; Al-Hasan <i>et al.</i> 2013) Kidney, fetal (Oh <i>et al.</i> 2008 <i>a</i> ) Bone Fetal (Lee <i>et al.</i> 2014) Postnatal (Lee <i>et al.</i> 2014)
Acute maternal hypoxia	1 h at 35, 40, 45, 50, 55 or 60 days' gestation; 7% O <sub>2</sub>	No	Placenta, fetal (Scheffen <i>et al.</i> 1990; Thompson <i>et al.</i> 2016) Brain, fetal (Mishra & Delivoria-Papadopoulos, 1988 <i>a,b</i> ; Mishra <i>et al.</i> 1988, 1995; Mishra & Delivoria-Papadopoulos, 1989, 1992; Graham <i>et al.</i> 1993, 1995; Lampley <i>et al.</i> 1995; Razdan <i>et al.</i> 1996; Maulik <i>et al.</i> 1998, 1999, 2001, 2002, 2005, 2008; Buonocore <i>et al.</i> 1999; Zanelli <i>et al.</i> 1999, 2005; Katsetos <i>et al.</i> 2001; Qayyum <i>et al.</i> 2001; Abedin <i>et al.</i> 2005; Vibert <i>et al.</i> 2008)
Maternal hypobaric hypoxia	For the last 17–28 days before term; 380–420 torr From 3 days post-conception; 3962 or 1600 m	Yes In 56 day cohort	Pulmonary vasculature, postnatal (Murphy <i>et al.</i> 1986) Placenta, fetal (Rockwell <i>et al.</i> 2000)
Maternal carbon monoxide exposure	200 ppm carbon monoxide for 10 h/day from day 23–25 of gestation until term	No, but smaller at 4 days after birth	Respiratory control, postnatal (McGregor <i>et al.</i> 1998)

(Continued)

Table 1. Continued

Model to induce developmental programming	Specific protocol	Altered fetal or neonatal growth	Organ system investigated (fetal/postnatal time point of analysis)
Unilateral uterine artery ligation	Performed at 28–32 days' gestation	IUGR in 26.5–50% of pups from ligated horn	Heart Fetal (Briscoe <i>et al.</i> 2004) Postnatal (Briscoe <i>et al.</i> 2004) Haemodynamics, fetal (Jones & Parer, 1983; Jansson <i>et al.</i> 1986; Detmer <i>et al.</i> 1991) Brain Fetal (Lafeber <i>et al.</i> 1984; Nitsos & Rees, 1990; Tolcos & Rees, 1997; Mallard <i>et al.</i> 1999; Diener & Rees, 2003, 2005; Tolcos <i>et al.</i> 2011; So <i>et al.</i> 2013; Chung <i>et al.</i> 2015; Tolcos <i>et al.</i> 2015) Postnatal (Thordstein & Kjellmer, 1988; Mallard <i>et al.</i> 2000; Rehn <i>et al.</i> 2004; Tolcos <i>et al.</i> 2011; So <i>et al.</i> 2013) Liver Fetal (Lafeber <i>et al.</i> 1984) Postnatal (Sarr <i>et al.</i> 2015) Adipose tissue, postnatal (Castaneda-Gutierrez <i>et al.</i> 2011; Sarr <i>et al.</i> 2014) Adrenal, kidney and aorta Fetal (Lafeber <i>et al.</i> 1984; Briscoe <i>et al.</i> 2004; Thompson <i>et al.</i> 2011a) Postnatal (Briscoe <i>et al.</i> 2004) Retina, fetal (Loeliger <i>et al.</i> 2008) Endocrine hormones, fetal (Jones <i>et al.</i> 1984, 1987; Carter <i>et al.</i> 2005) Vasculature, fetal (Canas <i>et al.</i> 2017; Herrera <i>et al.</i> 2017) Placenta, fetal (Herrera <i>et al.</i> 2016)
Progressive uterine artery occlusion	Ameroid constrictors implanted at 32–35 days' gestation	Yes	
Ablation of radial arteries supplying each placenta	Performed at 30–35 days' gestation	Yes	
Maternal nutrient restriction (global)	70% average food intake per kg of body weight of the control animals 4 weeks before conception until mid-pregnancy (35 days) increasing to 90% thereafter	Yes	Heart, fetal (Elias <i>et al.</i> 2016) Brain, fetal (Elias <i>et al.</i> 2016; Swanson <i>et al.</i> 2016) Liver, fetal (Olausson & Sohlstrom, 2003; Elias <i>et al.</i> 2016, 2017; Swanson <i>et al.</i> 2016) Heart and kidney, fetal (Elias <i>et al.</i> 2017) Adipose tissue, fetal (Kind <i>et al.</i> 2005) Placenta, fetal (Sohlstrom <i>et al.</i> 1998; Roberts <i>et al.</i> 2001a,b, 2002; Olausson & Sohlstrom, 2003; Elias <i>et al.</i> 2016, 2017)

(Continued)

**Table 1. Continued**

Model to induce developmental programming	Specific protocol	Altered fetal or neonatal growth	Organ system investigated (fetal/postnatal time point of analysis)
	F0 fed 0% of control food intake from gestational days 1–35 (early) or 36–70 (late); F1 females bred with control males	MNR late: yes; MNR early: F1 females heavier than control	Heart, F2 (Bertram <i>et al.</i> 2008) HPA function, F2 (Bertram <i>et al.</i> 2008)
	Deprived of all food for 48 h from 50 days' gestation	At 52 days' gestation, but no difference in birth weight	Brain Fetal (Lingas <i>et al.</i> 1999; Go <i>et al.</i> 2001; Chan <i>et al.</i> 2005) Postnatal (Lingas & Matthews, 2001) HPA function, postnatal (Lingas & Matthews, 2001)
	85% average food intake per kg of body weight of the control animals from at least 4 weeks before conception until fetal post-mortem or birth	Yes	Brain, fetal (Soo <i>et al.</i> 2012) Adipose tissue, fetal (Nguyen <i>et al.</i> 2010) Placenta, fetal (Soo <i>et al.</i> 2012) Blood pressure, postnatal (Kind <i>et al.</i> 2002) Glucose tolerance, postnatal (Kind <i>et al.</i> 2003) Cholesterol metabolism, postnatal (Kind <i>et al.</i> 1999) Muscle, fetal (Dwyer <i>et al.</i> 1995)
	Fed 60% of food intake of control in beginning, late or throughout pregnancy	Yes	Adipose tissue Fetal (Ashwell <i>et al.</i> 1987) Postnatal (Ashwell <i>et al.</i> 1987)
Maternal iron deficiency	Fed iron-deficient diet from 3 weeks prior to mating to PNS9	No	Cochlea, postnatal (Yu <i>et al.</i> 2016)
Maternal periconceptual overnutrition	160% food intake of control prior to mating and until 18 days' gestation	Greater percentage of larger and smaller fetuses	Vasculature, fetal (Krause <i>et al.</i> 2016)
Maternal high fat diet	Diet contained 40% energy from fat from mating, through pregnancy until weaning (PN21)	No difference in birth weight, but greater adiposity	Adipose tissue and insulin signalling, postnatal (Castaneda-Gutierrez <i>et al.</i> 2011)
	Diet containing either maize oil or beef dripping in last weeks of gestation	No	Brain, postnatal (Pavey & Widdowson, 1980) Adipose tissue, postnatal (Kirtland & Pavey, 1981)

(Continued)



Table 1. Continued

Model to induce developmental programming	Specific protocol	Altered fetal or neonatal growth	Organ system investigated (fetal/postnatal time point of analysis)
Antenatal glucocorticoid therapy	Dexamethasone (1 mg kg <sup>-1</sup> ) or vehicle on gestational days 40 and 41, 50 and 51, 60 and 61 (or until fetal post-mortem)	No	Brain and HPA function Fetal (Dean & Matthews, 1999; Liu <i>et al.</i> 2001; McCabe <i>et al.</i> 2001; Andrews <i>et al.</i> 2004; Setiawan <i>et al.</i> 2004; Baello <i>et al.</i> 2016; Iqbal <i>et al.</i> 2016) Postnatal (Banjanin <i>et al.</i> 2004; Owen & Matthews, 2007a,b; Setiawan <i>et al.</i> 2007; Dunn <i>et al.</i> 2010)
	Dexamethasone (1 mg kg <sup>-1</sup> ) or vehicle on gestational days 50 and 51	No	Brain and HPA function, postnatal (Dean <i>et al.</i> 2001)
	Betamethasone (1 mg kg <sup>-1</sup> ; phosphate-acetate mix) or vehicle on gestational days 40 and 41, 50 and 51, 60 and 61 (or until fetal post mortem)	No	Brain Fetal (McCabe <i>et al.</i> 2001; Owen & Matthews, 2003; Setiawan <i>et al.</i> 2004; Crudo <i>et al.</i> 2012, 2013a,b) Postnatal (Owen & Matthews, 2007a,b; Setiawan <i>et al.</i> 2007; Dunn <i>et al.</i> 2010; Crudo <i>et al.</i> 2012; Iqbal <i>et al.</i> 2012; Moisiadis <i>et al.</i> 2017) F2 (Crudo <i>et al.</i> 2012; Iqbal <i>et al.</i> 2012; Moisiadis <i>et al.</i> 2017) F3 (Moisiadis <i>et al.</i> 2017) Liver and kidney Fetal (Crudo <i>et al.</i> 2012) Postnatal (Crudo <i>et al.</i> 2012) F2 (Crudo <i>et al.</i> 2012) Placenta, fetal (Kalabis <i>et al.</i> 2009; Crudo <i>et al.</i> 2012) HPA function Postnatal (Owen & Matthews, 2007a,b; Dunn <i>et al.</i> 2010; Iqbal <i>et al.</i> 2012; Moisiadis <i>et al.</i> 2017) F2 (Iqbal <i>et al.</i> 2012; Moisiadis <i>et al.</i> 2017) F3 (Moisiadis <i>et al.</i> 2017)
Maternal stress	Exposure to high-frequency strobe light for 2 h at 50, 51 and 52 or 60, 61 and 62 days' gestation	No	Brain and HPA function, postnatal (Kapoor & Matthews, 2005, 2008, 2011; Kapoor <i>et al.</i> 2008, 2009)
	Exposed to 1 of 4 stressors every 2nd day in from 32 to 66 days' gestation and PN 1–25 (weaning)	No	Brain and HPA function, postnatal (Emack <i>et al.</i> 2008; Emack & Matthews, 2011)

(Continued)

**Table 1. Continued**

Model to induce developmental programming	Specific protocol	Altered fetal or neonatal growth	Organ system investigated (fetal/postnatal time point of analysis)
	Exposure to high-frequency strobe light for 2 h on gestational day 50, 55, 60 and 65	No	Brain and HPA function Fetal (Bennett <i>et al.</i> 2013) Postnatal (Bennett <i>et al.</i> 2015, 2017)
	F0 exposed to high-frequency strobe light twice a day during gestational days -7, 0, 7, 14, 21, 28, 35, 42; F2 generated from either F1 male/control female or F1 female/control male	No	Birth weight & placenta weigh, postnatal (Schopper <i>et al.</i> 2011) HPA function F1 (Schopper <i>et al.</i> 2012) F2 (Schopper <i>et al.</i> 2012)
Chronic maternal ethanol exposure	4 g ethanol per kg maternal body weight per day from 2 days' gestation until fetal post-mortem or 63 days' gestation	Some find IUGR, some do not	Brain, fetal (Cook <i>et al.</i> 1997; Iqbal <i>et al.</i> 2005, 2006; Hewitt <i>et al.</i> 2010, 2014) Liver, fetal (Hewitt <i>et al.</i> 2010) Behaviour, postnatal (Shea <i>et al.</i> 2012)
	3-4 g ethanol per kg maternal body weight per day for 5 days followed by 2 days without until term (~68 days)	No	Folate, fetal (Hewitt <i>et al.</i> 2011)
<i>In utero</i> infection (Group B Streptococci)	Wild-type GBS strain or an isogenic hyper-virulent, and hyper-haemolytic GBS strain (GBS $\Delta$ covR); 45 days' gestation	No	Bacterial invasion, fetal (Harrell <i>et al.</i> 2017)
Preterm birth (C-section)	C-section delivery at 62 days' gestation; 1 mg kg <sup>-1</sup> of betamethasone 24 and 12 h before delivery, neonates received 50 $\mu$ l of surfactant	No	Brain, postnatal (Kelleher <i>et al.</i> 2013; Palliser <i>et al.</i> 2015; Shaw <i>et al.</i> 2015) Microvasculature, postnatal (Dyson <i>et al.</i> 2012, 2014, 2015) Survival, postnatal (Cumberland <i>et al.</i> 2014) Placenta, fetal (Saif <i>et al.</i> 2016)

(Continued)

Table 1. Continued

Model to induce developmental programming	Specific protocol	Altered fetal or neonatal growth	Organ system investigated (fetal/postnatal time point of analysis)
Preterm birth (induced)	Induced at 62 days' gestation; aglepristone 10 mg kg <sup>-1</sup> 24 h prior to and on the morning of delivery, oxytocin 3 IU kg <sup>-1</sup> repeated in 30 min intervals until end of labour, betamethasone 1 mg kg <sup>-1</sup> 48 and 24 h before delivery	No	Growth and body composition, postnatal (Berry <i>et al.</i> 2015) Brain, postnatal (Shaw <i>et al.</i> 2016, 2017a)
Birth asphyxia	Induced by RU486 3 mg kg <sup>-1</sup> body weight at 55 and 56 days' gestation	No	Placenta, parturition (Gomez-Lopez <i>et al.</i> 2015)
	Asphyxia induced by clamping the umbilical cord at birth for 5 min	No	Brain, postnatal (Sanchez-Aparicio <i>et al.</i> 2008)
	Asphyxia induced by submerging in a water bath (37°C) for 2–4 min at birth	No	Brain, postnatal (Bernert <i>et al.</i> 2003)
Spontaneous fetal growth restriction	Variation in placental size and fetal nutrition due to spontaneous variation in litter size, birth to term	Yes, in litters of 6–7 body weight reduced by 22–38% compared to litters of 2	Fetal and placental weights, prenatal (Ibsen, 1928)
		Yes, in litters of 5 body weight reduced by 38% compared to litters of 1	Growth, body composition and appetite, postnatal (Horton <i>et al.</i> 2016)

high levels in human and guinea pig pregnancy, is a potent neurosteroid as well as an important precursor in the production of other neurotrophic steroids such as allopregnanolone. Intrauterine growth restriction (IUGR) and prenatal stress disrupt allopregnanolone production and result in reduced myelination, astrogliosis, altered GABA<sub>A</sub> receptor expression and behavioural deficits that are maintained in adolescence (McKendry *et al.* 2010; Kelleher *et al.* 2011; Bennett *et al.* 2013, 2015, 2017; Cumberland *et al.* 2017*b*). Similarly, preterm birth results in early loss of exposure to progesterone and its derivatives. The recent development of models of long-term survival following preterm birth in guinea pigs (Kelleher *et al.* 2013; Palliser *et al.* 2015; Shaw *et al.* 2015, 2016, 2017*a*) opens a critical area of developmental research in which the consequence of curtailed progesterone exposure and differences in substrate availability in preterm-delivered offspring compared to fetuses at the same postconceptional age can be explored.

Guinea pigs are a suitable for the investigation of long term behavioural and cognitive deficits following perinatal challenges. Commonly used assessments include the open field arena for quantification of exploration, locomotion, anxiety and hyperactive behaviours and social interactions (Iqbal *et al.* 2004; Kapoor & Matthews, 2005; Zipser *et al.* 2014; von Engelhardt *et al.* 2015; Shaw *et al.* 2016; Crombie *et al.* 2017; Cumberland *et al.* 2017*a*). The elevated plus maze (Rex *et al.* 1993; Crombie *et al.* 2017) and dark/light box (Zipser *et al.* 2014) are also used for measurements of anxiety. The Y-maze (Dobson *et al.* 2012*b*), modified Biel maze (Dobson *et al.* 2012*b*) and forced swim arena (Wicke *et al.* 2007) have been used in guinea pigs for measurement of behavioural inhibition, depressive states, spatial learning and memory deficits. In addition, the acoustic startle chamber (Rehn *et al.* 2004; Kapoor & Matthews, 2011) has been characterised for use in assessment of sensorimotor gating and attention deficits and the step tower (Zipser *et al.* 2014) to assess risk behaviours. Together, these tests provide key indicators of locomotor, emotional and cognitive disorders as well as risk-taking behaviour, social interaction and memory deficits. These tests can be paired with endocrine profiling using non-invasive salivary samples, which can readily be obtained from guinea pigs without inducing stress (Kapoor & Matthews, 2005; Kapoor *et al.* 2008).

Neurodevelopmental outcomes of IUGR in a number of animal species including guinea pigs has been extensively reviewed (Basiliou *et al.* 2015; Hunter *et al.* 2016). Clinically, infants born preterm exhibit reduced white matter volumes that have been linked to cerebral palsy in early premature infants and altered cognition- and emotion-based disorders such as anxiety and attention deficit hyperactivity disorder (ADHD) in moderate to late preterm infants (Bhutta *et al.* 2002; Linnet *et al.* 2006; Novik *et al.* 2006; Mulder *et al.* 2009; Johnson

*et al.* 2010; McLean *et al.* 2011). Guinea pig studies assessing prematurity and perinatal stress exposure have found similarly disrupted maturation of the oligodendrocyte lineage and reduced myelination, as well as dendrite and neurite developmental disruptions. As seen clinically, the deficits in white matter are commonly seen in the hippocampus, cortex, corpus callosum and cerebellum. Interestingly, postnatal studies have linked these disturbances to a number of behavioural deficits in exposed guinea pig offspring including increases in anxiety-like behaviours in female offspring and ADHD-like behaviours in male offspring (Bennett *et al.* 2016; Shaw *et al.* 2016; Cumberland *et al.* 2017*a*). The mechanism contributing to these adverse behavioural consequences remains unclear, but the overall process likely involves premature loss of placental factors that have sex-dependent trophic effects on late gestation neurodevelopment. These findings suggest that a replacement approach may improve long-term neurodevelopmental outcome. Current studies are evaluating neuroprotective benefits of replacing key placentally derived steroids, specifically progesterone and its neuroactive metabolite allopregnanolone, which are lost following preterm birth or reduced during times of stress using the guinea pig (Palliser *et al.* 2015; Crombie *et al.* 2017; Shaw *et al.* 2017*b*).

### Programming of the HPA axis and stress-related behaviours

The effects of excess glucocorticoids (maternal glucocorticoid exposure and maternal stress in pregnancy) on the programming of the HPA axis and stress-related behaviour has been investigated using the long-term programming effects of synthetic glucocorticoid (sGC) administration and chronic and acute maternal prenatal stress (for review see Moisiadis & Matthews, 2014*a,b*). From these studies, it has become clear that prenatal glucocorticoid exposure and maternal stress lead to profound long-term changes in basal and activated HPA function as well as modified behaviours. The phenotypes are driven by dramatic changes in gene expression in the brain, pituitary and adrenal, which in turn appear to involve complex changes in epigenetic regulation.

The nature of these effects is highly dependent on the timing of exposure, sex and age of offspring, and in females the stage of reproductive cycle at which phenotype is assessed. Prenatal stress at GD50 resulted in increased anxiety behaviour in juvenile male offspring, and reduced ambulatory activity, reduced attention and elevated basal HPA activity in adult male offspring (Kapoor & Matthews, 2005, 2011). In contrast, prenatal stress at GD60 resulted in an increased cortisol response to stress in male adult offspring (Kapoor & Matthews, 2005). Interestingly the behavioural affects appeared to be modulated at least in part by testosterone.

In adult female offspring, prenatal stress at GD60, but not GD50, resulted in lower basal cortisol levels (Kapoor & Matthews, 2008). Additional evidence that timing of exposure is critical to outcome is illustrated by the fact that prenatal stress at GD50 decreased spatial learning in adult male offspring, whereas the same stressor delivered at GD60 increased spatial learning (Kapoor *et al.* 2009). The effects of prenatal stress on programming of HPA function are sensitive to reproductive cycles in females, and the relatively long reproductive cycle in this species allows testing within each phase. Prenatal stress at GD50 or GD60 reduced the cortisol stress response during the oestrous phase, but not during the luteal phase of the cycle, in adult female offspring (Kapoor & Matthews, 2008). Effects of glucocorticoid exposures are age- and well as sex-dependent. Exposure to sGC (betamethasone) during late gestation resulted in decreased cortisol response to a stressor in juvenile male offspring (Owen & Matthews, 2007a). On the other hand, sGC (dexamethasone) exposure resulted in a reduction in basal and stress-induced cortisol levels in young adult male offspring (Liu *et al.* 2001). Interestingly, this effect was no longer observed in older males (Banjanin *et al.* 2004). In adult females, prenatal sGC (betamethasone or dexamethasone) exposure resulted in reduced basal HPA activity only in the luteal phase and increased basal HPA activity only in the oestrous phase of the reproductive cycle (Liu *et al.* 2001; Dunn *et al.* 2010). Finally, single *versus* multiple courses of sGC exposure have different effects on offspring HPA function. A single course of sGC in late gestation led to a reduced cortisol stress response in juvenile males (Dean *et al.* 2001), but in contrast, exposure to multiple courses of sGC led to decreased stress response in juvenile male offspring (Owen & Matthews, 2007a).

**Transgenerational effects of sGC.** Programming studies in the guinea pig have been extended to demonstrate transgenerational effects of fetal exposures to sGC and nutrient restriction on HPA function and behaviour, via both maternal and paternal transmission. Prenatal sGC exposure resulted in a blunted cortisol response to stress in male and female F2 offspring of treated mothers (i.e. maternal transmission), as well as altered activity in an open field (Iqbal *et al.* 2012). In addition, F2 males exhibited increased negative feedback sensitivity to glucocorticoids while females showed decreased feedback sensitivity (Iqbal *et al.* 2012). These effects are potentially due to the direct effects of sGC exposure on F2 gametes in F0 females, gametic (genetic) selection, or transgenerational transmission by other mechanisms such as epigenetic modifications of DNA methylation. Accumulating evidence points to the latter explanation. In a recent study, prenatal sGC treatment led to altered cortisol responses to stress and behaviours in female and male juvenile F2 and

F3 offspring from both parental lines, demonstrating maternal and paternal transgenerational programming to F3 (Moisiadis *et al.* 2017). Interestingly, the endocrine and behavioural phenotypes were strongest in juvenile females following paternal transmission, strongly implicating transgenerational epigenetic transmission. Also demonstrating transgenerational programming, maternal undernutrition changed guinea pig heart structure and HPA function across two generations (Bertram *et al.* 2008). F1 male offspring showed elevated blood pressure, as well as increased thickness and mass of the left ventricular wall. These changes in the heart structure were carried over to the F2 male offspring. Maternal undernutrition also increased basal cortisol and altered HPA response to a stress challenge in both F1 and F2 offspring.

### Programming of cardiovascular function

**Cardiac response to stress.** The maturity of the cardiovascular system of the fetal guinea pig is an advantage over other species for studying the effects of *in utero* stress on fetal heart development and the programming effects that ensue after birth. Studies have identified hypoxia-induced increased gene/protein expression in inducible nitric oxide synthase (NOS) in fetal guinea pig heart ventricles (Thompson & Dong, 2005; Dong & Thompson, 2006; Thompson *et al.* 2009; Evans *et al.* 2012a) and increased expression (Dong & Thompson, 2006) and vasodilator contribution of endothelial NOS in the coronary circulation (Thompson *et al.* 2000), all of which will programme poor heart health after birth. Prenatal hypoxia increases other signalling factors such as the generation of lipid peroxide products (malondialdehyde) (Evans *et al.* 2012a), proinflammatory cytokines (TNF $\alpha$ , IL-6, IL-1b) and matrix metalloproteinases (MMP2 and 9) (Oh *et al.* 2008b) in cardiac left ventricles of the term fetal guinea pig (Fig. 6). This may be mediated by oxidative stress via generation of reactive oxygen species as evidenced by the inhibitory effect of maternal administration of the antioxidant *N*-acetylcysteine. Malondialdehyde levels of the fetal guinea pig heart were elevated under conditions of chronic intrauterine hypoxia and reversed to normoxic levels in the presence of *N*-acetylcysteine (Evans *et al.* 2012a; Al-Hasan *et al.* 2013). In addition, maternal *N*-acetylcysteine reversed the hypoxia-induced increase in fetal cardiac MMP9 (Evans *et al.* 2012a) protein levels and the decrease in fetal cardiac mitochondrial cytochrome *c* oxidase activity (Al-Hasan *et al.* 2013). The decreased cytochrome *c* oxidase activity of hypoxic fetal guinea pig ventricles was sustained in hearts of prenatally hypoxic guinea pig offspring, suggesting mitochondrial programming in offspring hearts (Al-Hasan *et al.* 2014).



**Vascular function.** Guinea pigs are born with both functional and well-differentiated adrenergic nerves in the peripheral tissues (O'Donnell & Saar, 1975) comparable to humans (Armati-Gulson & Burnstock, 1983). This is particularly important since emerging data suggests that ex-preterm offspring, particularly females, have altered autonomic regulation of the cardiovascular system (Berry *et al.* 2013; Kim *et al.* 2014), which may contribute to their increased cardiovascular risk. Comparative studies in humans and guinea pigs have demonstrated similar profiles of vascular transition in both term- and preterm-born newborns. In preterm animals born at GD62, as in preterm humans born prior to 29 weeks' completed gestation, there is a period of high microvascular flow after birth, associated with central cardiovascular compromise, morbidity and mortality (Stark *et al.* 2008; Dyson *et al.* 2012, 2014) providing a model for studying cardiovascular transition at birth in preterm newborns, and the contribution of early microvascular compromise to adult cardiovascular disease in the ex-preterm adult. Human studies now consistently demonstrate higher blood pressure in ex-preterms than in controls (Hack *et al.* 2005), especially in females (Bonamy *et al.* 2005). Furthermore, this is associated with lower skin capillary density (Bonamy *et al.* 2007) and retinal microvascular changes (Kistner *et al.* 2002), which highlights the need for an appropriate animal model to interrogate

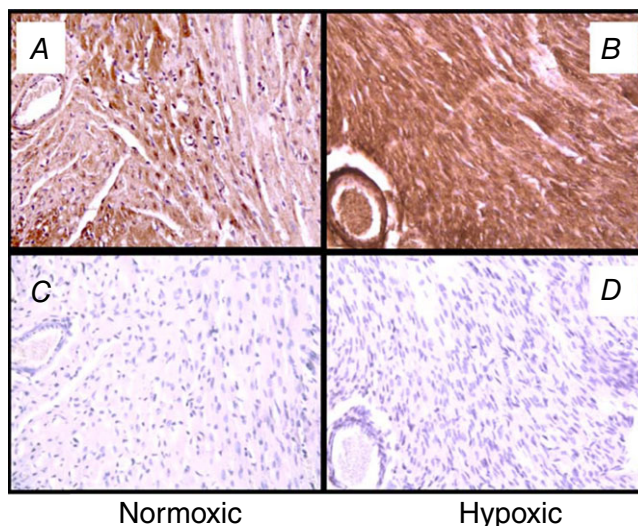
the vascular mechanisms contributing to increased cardiovascular disease risk in the ex-preterm.

As in humans, there is increased mean arterial blood pressure in adult rodents including guinea pigs that were growth restricted by experimentally induced reductions in uterine blood flow (Persson & Jansson, 1992; Battista *et al.* 2002). These changes in blood pressure are also associated with peripheral endothelial dysfunction and vascular remodelling in the aorta early in the neonatal period with progressively worsening impairment throughout life in human studies of IUGR (Zydzorczyk *et al.* 2017). Similarly, low birth weight adult guinea pigs have increased stiffness in conduit arteries (Thompson *et al.* 2011b) along with a decreased NOS-mediated relaxation that is not further impaired by the exposure to an obesogenic diet (Thompson *et al.* 2014). This is preceded by fetal vascular changes. Aortae from growth-restricted guinea pig fetuses show a decreased elastic lamina (Thompson *et al.* 2011b) as well as an increased stiffness, contractile force and media thickness (Canas *et al.* 2017). These changes are also observed in femoral resistance arteries (Canas *et al.* 2017). Additionally, these fetal vessels have a decreased NO-dependent relaxation that is mainly related with an impaired endothelial function (Herrera *et al.* 2017). This model identifies signs of endothelial dysfunction in aorta, femoral and umbilical arteries after poor growth *in utero* (Herrera *et al.* 2016, 2017), findings that can be translated to clinical practice to predict systemic vascular dysfunction in the long term based on umbilical vascular impairment.

Studies in placental and umbilical vessels represent an important source of data for DOHaD that can be difficult to collect, especially in human. Studies in umbilical arteries from normal weight and growth restricted guinea pig fetuses show remarkable similarities with human umbilical arteries (Bruch *et al.* 1997; Burkhardt *et al.* 2009; Krause *et al.* 2013a) in their *ex vivo* vascular responses and remodelling. These differences between normal weight and growth restricted guinea pig fetuses include an impaired endothelial function (Herrera *et al.* 2017), reduced media thickness and decreased contractile force (Canas *et al.* 2017).

### Programming of the lung

To date, limited studies have exploited the similarities in the temporal development of the lung between guinea pigs and humans. Guinea pigs delivered 'late preterm' (GD65/68; 96% completed gestation) require respiratory support (Berry *et al.* 2015) and exhibit transient respiratory distress, with evidence of acute lung injury (atelectasis, pulmonary oedema, fibrin deposition and inflammatory cell infiltration) 96 h after birth. This injury is exacerbated when preterm animals are exposed to high oxygen concentration (95% O<sub>2</sub>), and/or high tidal



**Figure 6.** Immunostaining of matrix metalloproteinase 9 (MMP9) of normoxic (A and C) and 14 day hypoxic (B and D) fetal guinea pig hearts

Positive immunostaining (brown stain) for MMP9 was localized in both cardiac tissue and blood vessels. Negative controls were generated in the absence of the primary antibody to MMP9 protein (C and D). Sections were counterstained with haematoxylin QS (Vector Laboratories). Original magnification:  $\times 200$ . From Oh *et al.* (2008b) with permission.

volume ventilation strategies as frequently experienced by neonates being resuscitated and receiving respiratory support in the NICU (Koshy *et al.* 2011). Together with similar timing of lung maturation between guinea pigs and humans (Sosenko & Frank, 1987; Kelly *et al.* 1991a), these similar acute outcomes in guinea pigs suggest that this species may be a suitable model for studying the long-term consequences of the lung injury that follows preterm birth.

### Programming of renal function

As is the case with human development, nephrogenesis is completed in late gestation in the guinea pig. Perinatal insults such as fetal growth restriction and preterm birth disrupt nephrogenesis, leading to altered renal structure and function in later life in both humans (Newsome *et al.* 2017) and guinea pigs (Briscoe *et al.* 2004). With the increasing burden of morbidity posed by chronic kidney disease, work in guinea pigs is ideal to assess the mechanisms linking perinatal insult to later morbidity as well as to assess the potential efficacy of therapeutic interventions in early or later life.

### Programming of metabolic outcomes

A suitable animal model is needed to investigate the mechanisms underpinning the range of programmed metabolic dysfunction described in humans including obesity, insulin resistance, and perturbed cholesterol and glucose metabolism. Muscle development is extensively prenatal, similar to the human, and thus is vulnerable to developmental programming. Altered development of these metabolic tissues in the guinea pig fetus has been demonstrated in response to a perturbed *in utero* environment. For example, maternal feed restriction reduces relative weight of the fetal biceps brachii muscle (Dwyer *et al.* 1995; Kind *et al.* 2005), and reduces fibre numbers, particularly secondary fibres, in the biceps (Ward & Stickland, 1991; Dwyer & Stickland, 1992; Dwyer *et al.* 1995). This effect of maternal undernutrition on fetal muscle development differs between muscles, with fibre numbers in soleus muscle, a muscle with more slow-twitch Type 1 fibres, not affected (Ward & Stickland, 1991; Dwyer & Stickland, 1992). Further postnatally, insulin sensitivity is reduced in low birth weight pups and the percentage of body weight is negatively correlated with birth weight (Davis & Auten, 2010).

Maternal feed restriction also reduces relative weight of fetal liver, but in contrast, increases relative weight of interscapular and retroperitoneal fats in GD60 fetuses (Kind *et al.* 2005). Adipocyte populations in fetal adipose tissue are also altered with an increased proportion of large lipid locules in the perirenal depot (Nguyen *et al.* 2010) and increased proportion of unilocular adipocytes in the

interscapular depot, suggesting sparing of white adipose tissue at the expense of brown adipose tissue in the fetus of feed restricted mothers (Kind *et al.* 2005).

**Postnatal growth and body composition.** A number of studies, with differing prenatal or perinatal insults, have also reported altered postnatal adipose tissue development. Guinea pigs have been proposed as a good model for studying the effects of maternal obesity because the overnutrition results in increase adipose stores and decreasing fertility and litter size (Michel & Bonnet, 2012). Young adult males with low birth weight have increased relative epididymal fat weight, independent of changes in whole body adiposity, but accompanied by adipocyte hypertrophy, increased lipid storage and altered expression of genes involved in lipid metabolism in the visceral epididymal depot (Sarr *et al.* 2014). Similarly, relative retroperitoneal fat weight is increased in young adult male offspring of feed-restricted mothers (Kind *et al.* 2003). Chronic prenatal ethanol exposure throughout gestation decreases birth weight, but increases total, visceral and subcutaneous adipose volumes, assessed by MRI, in young adult guinea pigs (Castaneda-Gutierrez *et al.* 2011; Dobson *et al.* 2012a). A high fat diet during pregnancy and lactation also increases the percentage of body fat in offspring at 2 and 21 days of age, and following weaning onto a high fat diet increases relative weight of retroperitoneal fat in 145-day-old offspring (Castaneda-Gutierrez *et al.* 2011). Other body fat depots did not differ in these adult offspring of fat-fed mothers, and these depot-specific effects of prenatal environment may reflect differences in the extent to which the fat depots undergo hyperplasia before birth in the guinea pig (Castaneda-Gutierrez *et al.* 2011).

Changes in adiposity in young adult guinea pigs may also relate, at least in part, to alterations in feed intake. Relative energy intake from weaning to day 60 is higher in low birth weight male guinea pigs (Sarr *et al.* 2014). Similarly, feed intake is increased during the juvenile period (day 40–60) in IUGR offspring from larger litters. Offspring from larger litters also exhibit increased fractional growth rates in both the neonatal and juvenile periods, suggesting a change in partitioning of nutrients towards growth, and consistent with this, neonatal catch-up growth predicts increased visceral adiposity in male offspring (Horton *et al.* 2016). Differences in postnatal growth, with low-birth-weight guinea pigs attaining similar weights to their non-growth retarded counterparts, is also reported in other studies (Dobson *et al.* 2012a; Sarr *et al.* 2014; Nevin *et al.* 2018), including neonatal catch-up growth (Dobson *et al.* 2012a; Nevin *et al.* 2018), which also occurs in humans. Other perinatal compromises also alter postnatal growth trajectories. For example, between term equivalent age and weaning ex-preterm animals demonstrate increased

weight and ponderal index relative to term-born pups (Berry *et al.* 2015).

**Cholesterol metabolism.** Guinea pigs resemble humans in that they carry the majority of their cholesterol in low density lipoprotein (LDL), have a similar moderate level of hepatic cholesterol synthesis and catabolism, have plasma cholesterol ester transfer protein, lecithin: cholesterol acyltransferase and lipoprotein lipase activity, and exhibit similar sex-based differences in plasma lipoprotein profiles (Fernandez & Volek, 2006; Fernandez, 2008). The guinea pig is also an appropriate model for studying hypercholesterolaemia (Aggarwal *et al.* 2006) and atherosclerosis (Fernandez & Volek, 2006; Mangathayaru *et al.* 2009) because of its moderate plasma cholesterol response and normal triglyceride response to a high fat–high cholesterol diet (deOgburn *et al.* 2012), and is a model for statin-induced cholesterol lowering and myotoxicity (Madsen *et al.* 2008). Total plasma cholesterol is higher in male offspring of feed-restricted mothers both before and after a 6-week challenge with high dietary cholesterol (Kind *et al.* 1999). When offspring were divided according to birth size, plasma cholesterol levels did not differ when fed a standard diet, but low-birth-weight guinea pigs had higher total and LDL cholesterol levels when challenged with a high cholesterol diet (Kind *et al.* 1999).

**Insulin sensitivity and glucose tolerance.** The guinea pig provides a unique model to explore developmental programming of insulin-regulated glucose metabolism, since its insulin is immunologically distinct from that of other species (Watt, 1985), which allows exogenous and endogenous insulin to be differentiated in assays. Because the insulin receptor of guinea pigs is less divergent from that of other species than the insulin molecule itself (Muggeo *et al.* 1979), glucose uptake can be stimulated by administering readily available insulin from other species, including recombinant human insulin. Hyperinsulinaemic euglycaemic clamps can be performed repeatedly in guinea pigs, and we have used this approach to show dose-dependent insulin-stimulated glucose uptake in this species (Horton *et al.* 2017). Although sensitivity to human insulin is lower in guinea pig than in humans, maximal glucose-uptake responses are similar in both species (Horton *et al.* 2017). Guinea pig models of type 2 diabetes based on high-fat–high-carbohydrate feeding have been developed, and exhibit impaired glucose tolerance and compensatory hyperinsulinaemia (Podell *et al.* 2017). There is some evidence for programming of glucose metabolism by maternal nutrition in guinea pigs, as occurs in humans (Ravelli *et al.* 1998). Fasting insulin levels are increased and glucose tolerance is impaired in young adult male offspring of moderately feed-restricted guinea pigs, when

compared to offspring of mildly restricted mothers (Kind *et al.* 2003). When divided according to weight at birth, low-birth-weight males exhibit fasting hyperinsulinaemia, suggestive of insulin resistance, but no impairment of glucose tolerance compared to control offspring at this age (Kind *et al.* 2003). This area is relatively underexplored, however, with few studies having assessed developmental programming of glucose metabolism in the guinea pig, including at later adult ages when earlier insulin resistance may result in development of impaired glucose tolerance. Alterations in pancreatic  $\beta$ -cell area and hepatic expression of insulin signalling genes occur in adult offspring following chronic prenatal ethanol exposure (Dobson *et al.* 2012a); however, the programming of insulin sensitivity and its tissue-specific determinants in the guinea pig are an area requiring further study.

### Programming of reproductive function

The physiological similarities in reproductive physiology between guinea pigs and humans make them an ideal experimental model to explore the reproductive consequences of an altered perinatal environment. For instance, postnatal growth is impacted by prenatal exposures, and body weight of non-pregnant adult females correlates positively with number of corpora lutea (Eckstein & McKeown, 1955) as well as their subsequent litter sizes when mated (Horton *et al.* 2016); it is therefore likely that reproductive outcomes in females are programmed by many prenatal factors than modify fetal growth. Programming of male fertility and its determinants is a comparatively understudied field. However, some evidence for developmental programming of male reproduction has been reported for *Cavia aperea*, the ancestor of domestic guinea pigs, *Cavia porcellus*. In *Cavia aperea*, males whose mothers were exposed to unstable social environments (fortnightly regrouping with novel animals) throughout pregnancy and lactation had different developmental profiles of testosterone during development than F1 males from control dams (unchanged social group throughout pregnancy and lactation), including higher testosterone in early postnatal life (Siegeler *et al.* 2013). Other reproductive parameters including testis and accessory gland weights, sperm ploidy, motility and DNA fragmentation in the F1 males as adults at 107 days of age were, however, unchanged (Siegeler *et al.* 2013).

There is also direct, albeit limited, evidence for transmission of reproductive programming through both female and male lines in *Cavia porcellus*. Maternal undernutrition in early (days 1–35) or late (days 36–70) pregnancy did not affect age at first oestrous or mating in their F1 daughters, i.e. the generation that was *in utero* during maternal exposure (Bertram *et al.* 2008). However, daughters of dams that were nutrient restricted



in late pregnancy took twice as long to become pregnant as daughters of unrestricted dams, with no effect of maternal undernutrition in early pregnancy on F1 age at first pregnancy (Bertram *et al.* 2008). Birth weight of male and female progeny was reduced at both F1 and F2 by late pregnancy undernutrition in the F0 dams (Bertram *et al.* 2008). Prenatal exposure to repeated maternal courses of the synthetic corticosteroids betamethasone or dexamethasone in the second half of gestation also did not alter the timing of reproductive maturation (first oestrous), nor did these affect cycle length (Dunn *et al.* 2010). Intriguingly, although betamethasone-exposed F1 females took about twice as many cycles to conceive as controls, similar to effects of F0 pregnancy undernutrition, neither glucocorticoid altered litter size, gestation length or litter average birth weight in F1 pregnancies (Dunn *et al.* 2010). F0 repeated glucocorticoid exposure, in this case dexamethasone and not betamethasone, also altered sex balance at birth of the F2 generation, with a higher proportion of females in the F2 litters of F1 dams exposed to dexamethasone *in utero* (Dunn *et al.* 2010). In recently reported studies of transgenerational effects in this model, the lack of effect of repeated F0 betamethasone courses on reproductive parameters including age at conception, gestation length, litter size and sex ratio was confirmed for pregnancies in F0, F1 and F2 females in the maternal line (Moisiadis *et al.* 2017). Intriguingly F2 daughters of F1 sons from sGC-exposed pregnancies did tend to have longer gestations than F2 daughters of control F1 males (Moisiadis *et al.* 2017).

### Role of epigenetics in DOHaD and use of -omics data in guinea pigs

Epigenetics plays a crucial role in programming. Epigenetic mechanisms include DNA methylation/demethylation, histone post-transcriptional modification, chromatin remodelling and non-coding RNA. Recently, it has been demonstrated that fetal growth restriction in guinea pig fetuses is associated with changes to the DNA methylation pattern in the *nos3* (eNOS) gene promoter (Herrera *et al.* 2017). This altered DNA methylation pattern occurs in endothelial cells from the aorta, umbilical and femoral arteries. Comparable findings have been reported in human umbilical artery endothelial cells from pregnancies affected by fetal growth restriction (Krause *et al.* 2013b).

There is accumulating evidence that epigenetic mechanisms are involved in the programming of long-term endocrine and behavioural outcomes following prenatal glucocorticoid exposure. Studies have examined activation of the glucocorticoid receptor and its binding to glucocorticoid response elements that regulate a large number of genes. These studies have utilized multi-dimensional omics analyses of mRNA with gene

expression array, glucocorticoid receptor binding with chromatin immunoprecipitation and microarray hybridization (ChIP-on-chip), and DNA methylation with immunoprecipitation followed by qPCR (mDIP-qPCR) (Crudo *et al.* 2012, 2013a,b). The endogenous glucocorticoid surge in late gestation as well as sGC exposure has a substantial impact on the hippocampal transcriptome, glucocorticoid receptor–DNA binding and the DNA methylation landscape in the fetal hippocampus at 24 h and 14 days following the sGC exposure. These data support the hypothesis that glucocorticoid exposure in late gestation plays a significant role in modifying the transcriptional and epigenetic landscapes of the developing fetal and juvenile hippocampus (Crudo *et al.* 2013a). Transcription factor binding has been mapped at promoters and enhancers associated with active transcription in adult hippocampi. Furthermore, reduced representation bisulfite sequencing and ChIP-sequencing has been undertaken to examine the DNA methylation status of genomic regions that overlap with RNAPolII at transcription start sites in promoters and H3K4me at enhancer regions (Boureau *et al.* 2018). Based on this map, a candidate gene approach has been utilized to assess DNA methylation levels using DNA capture to measure the effects of sGC during development. Recently, the same group have shown that sGC exposure during late gestation programmed transgenerational gene expression changes in the paraventricular nucleus of the hypothalamus using RNA-seq (Moisiadis *et al.* 2017). These alterations included gene networks for type II diabetes, thermoregulation and collagen formation. Taken together, the guinea pig serves as an excellent model not only because it shares similar patterns of brain development with the human but also because it is accessible for the use of transcriptomic, genomic and epigenomic approaches.

### Future directions for developing DOHaD interventions using guinea pig models

Pregnant guinea pigs have been used to study the effects of maternal infection on fetal development, such as by cytomegalovirus and listeria, because of their similar susceptibility and clinical outcomes to humans (Wadhwa Desai & Smith, 2017). This commonality between the human and guinea pig immune responses has also been applied to the development of vaccines for tuberculosis (Dey *et al.* 2009, 2011; Jain *et al.* 2011). However, compared to mice and rat models of DOHaD, guinea pigs have been relatively underutilised as a preclinical model in which to develop potential therapeutic fetal interventions. The reasons for this are likely to be multifactorial and pragmatic: the benefits of guinea pig models in terms of their long gestational age length, relatively small pup number and DOHaD outcomes are likely to have been underappreciated. Published studies of drug or surgical

interventions in guinea pig pregnancy are few in number. Regulatory authorities currently lack familiarity with data from guinea pigs for decisions about novel therapeutics; this lack of familiarity will inevitably influence decisions about the choice of specific animal models (Swanson & David, 2015).

Despite their physiological advantages for DOHaD studies, guinea pigs are rarely used for reproductive toxicology studies of new drugs. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines govern reproductive toxicity studies in women and men (ICH, 2017). They specify that any programme should allow exposure of the novel chemical to all stages of development throughout one complete life cycle: for example, from conception in one generation to conception in the following generation. In practice, a number of overlapping studies are conducted to cover fertility and early embryonic development, pre- and post-natal development including lactation and weaning, and embryo–fetal development (Baldwin, 2009). Fertility and pre- and postnatal development studies need only be conducted in one mammalian species, which, primarily for logistical reasons, is usually the rat. For embryonic and fetal development two mammalian species are tested: one should be a rodent, often the rat for pragmatic reasons, and the other a non-rodent, usually the rabbit, where there is a large body of historical data for comparison. Despite this historical precedent, the guinea pig affords a species that has a high degree of commonality in placentation and fetal developmental trajectory to humans; thus, we suggest that the increased housing and care costs of guinea pigs compared to rats are more than offset by their advantages as a superior model for pregnancy-related drug development studies.

Some of the most challenging and complex areas of clinical research lie in understanding the late effects of adverse pregnancy outcome, especially preterm birth and fetal growth restriction. Robust guinea pig models have been developed that rely on the similarities between the guinea pig and human, across key developmental, neuroendocrine and metabolic characteristics, to mirror the physiological phenotype of human patients. These models are an important tool for advancing our understanding of the mechanisms underpinning the later pathophysiological complications of adverse pregnancy outcome, but will also enable the efficacy and safety of new therapeutics to be developed in a species with huge direct translational potential.

### Therapeutic interventions to prevent/correct programming

Early studies investigated the effect of exercise on the adult guinea pig as a tool to evaluate cardiopulmonary

function (Yilmaz *et al.* 2008), plasma and hepatic lipid profiles (Ensign *et al.* 2002), amino acid incorporation into skeletal muscle (McManus *et al.* 1975; Rogers *et al.* 1979) and hepatic glucose production in denervated livers (Wiersma *et al.* 1995), the latter because of rich liver innervation comparable to humans and in contrast to rat and dog. Treadmill exercise increased ventilation and CO<sub>2</sub> output linearly with oxygen uptake, increased lung diffusing capacity with respect to pulmonary blood flow as well as membrane diffusing capacity and pulmonary capillary blood volume (Yilmaz *et al.* 2008). The functional recruitment of alveolar microvasculature with exercise was similar to that measured in dog and human (Yilmaz *et al.* 2008). Exercise improved plasma lipid profiles in adult guinea pigs with 33% lower triacylglycerol, 66% higher HDL cholesterol and 31% lower plasma free fatty acid levels compared to rest, similar to the effects on lipid profiles in humans (Ensign *et al.* 2002). In newborn guinea pigs, treadmill exercise of 1–3 weeks had no effect on lung growth or alveolar multiplication, indicating that the guinea pig alveoli are fully developed at the time of birth (Ross & Thurlbeck, 1992), different from rat or mice whose alveoli are absent at birth (Thurlbeck, 1975). Only two studies using the pregnant guinea pig have reported the effects of maternal exercise on the fetus (Nelson *et al.* 1983; Smith *et al.* 1983), although other species such as the sheep have been used (Lotgering *et al.* 1983). Maternal exercise of pregnant guinea pigs decreases fetal weight, kidney weight, placental weight and placental diffusing capacity (Nelson *et al.* 1983). The decrease in diffusing capacity is directly related to the maternal surface exchange area of the labyrinth and the total surface area of the placenta (Smith *et al.* 1983). This suggests that maternal exercise can reduce fetal weight by compromising placental development.

Prenatal interventions aimed at improving DOHaD outcomes have already been successfully evaluated in guinea pig pregnancy. Maternal treatment with the antioxidant *N*-acetylcysteine (500 mg kg<sup>-1</sup> day<sup>-1</sup>) restored fetal growth following experimentally induced growth restriction (Herrera *et al.* 2017). Similarly, maternal sildenafil (50 or 500 µg kg<sup>-1</sup> day<sup>-1</sup>) from mid-gestation until delivery protected pups against induced asphyxia at birth in a dose-dependent manner, and the higher sildenafil dose increased fetal pup weight at term (Sanchez-Aparicio *et al.* 2008) compared to untreated growth-restricted pregnancies. Both *N*-acetylcysteine and sildenafil are now being considered for the treatment approaches of fetal growth restriction in human pregnancy.

Targeting the uteroplacental circulation can be achieved in guinea pig pregnancy through laparotomy and external application of thermolabile pluronic gel containing therapeutic drugs to exposed uterine arteries and radial arteries (Mehta *et al.* 2016). Direct injection of the uterine arteries was associated with high fetal loss, while upstream



**Table 2. Gaps in our knowledge that can be filled using guinea pig models**

Current uses of guinea pig models in DOHaD	What the guinea pig model can offer in future DOHaD studies
DOHaD models of common, clinically important conditions have been established in guinea pigs that result in similar outcomes as in humans	The commonality in physiology between guinea pigs and humans can be exploited much more aggressively in studies of reproductive toxicology, maternal medication exposure and therapeutic fetal interventions
Safety and efficacy studies of novel therapeutic interventions have been tested in guinea pigs and their results used to inform the development of human studies	Guinea pigs can enable assessments of long term outcomes, including multi- and trans-generational outcomes as well as a rigorous assessment of the paternal contribution to DOHaD

injection of the internal iliac arteries did not target the uteroplacental circulation. Applying an adenoviral vascular endothelial growth factor vectors in combination with pluronic gel led to local transgenic vascular endothelial growth factor protein expression in transduced arteries, altered vascular reactivity and increased fetal growth in global maternal nutrient restriction FGR pregnancies (Swanson *et al.* 2016). In addition, atrial natriuretic peptide infusion selectively increased blood flow to placentas of FGR fetuses while placental blood flow of normal-sized fetuses remained unchanged (Jansson, 1992). Many other exciting and novel therapeutic interventions to support cardiometabolic and neurodevelopmental health in preterm and/or growth-restricted pups are also under evaluation as the awareness of guinea pigs as a translational model for DOHaD research continues to increase (Table 2).

### Limitations of guinea pigs as a model of DOHaD

Despite the many strengths of guinea pig models in DOHaD outlined in this review, it is important to note that there are some limitations. Guinea pigs are difficult to chronically catheterise and therefore it is difficult to invasively monitor and manipulate their intrauterine environment; such studies are more easily carried out in fetal sheep. Having made this point, elegant studies by Peeters's team used indwelling catheters to show fat uptake by the uterus and the fetus (Peeters *et al.* 1984, 1986). Molecular biology techniques are less developed than in mice and rats, requiring custom rather than 'off the shelf' solutions. It is feasible to design and optimize primers for gene expression studies, but there are instances where there are no available antibodies to measure protein abundance. Not all outcome measures that can be made in rats and mice can be currently made in guinea pigs; however, as more researchers with different interests and expertise enter the area, these experimental tools will be developed as we have seen with gene expression, cardiovascular function and behavioural testing to date. These limitations highlight the need for appropriate selection

of animal models to answer each scientific question. The longer gestation length, time to F2 and F3 generations and husbandry requirements needed to maintain a guinea pig colony mean that guinea pig studies will inevitably cost more to run than those in rats or mice. However, guinea pig studies are still considerably cheaper to run than large animal or non-human primate studies, with significant translational potential as described above to justify these slightly higher costs.

Guinea pigs are multiparous, producing more than one offspring at a birth. Litter-bearing models of pregnancy or fetal studies can introduce an increased level of complexity to the statistical design of a study (Festing, 2006). With all litter-bearing species, litter effects can be introduced when females, whole litters or siblings within a litter are assigned to the same group (Festing, 2006). The litter is the experimental unit, not the individual pup (Williams *et al.* 2017) and thus an adequate number of dams must be used. Additionally, the use of more advanced statistical methods such as restricted (or residual, or reduced) maximum likelihood (REML), mixed effects models and nested analysis can take into account a range of factors like maternal diet, weight, litter size, pup weight and sex within the statistical model (Wainwright *et al.* 2007). Employing these techniques will reduce intra-litter variation making for more robust statistical interpretation during analysis of litter-bearing animal models of pregnancy and development. For example, it would not be appropriate to use more than one pup of each sex at one time point in an analysis without nesting for litter (Lee *et al.* 2014).

### Conclusion

In conclusion, the guinea pig is a versatile species in which the key facets of human pregnancy and fetal development are mirrored both in health and following clinically relevant perturbation. The relatively short life cycle, modest housing and husbandry requirements and docile temperament make it an ideal species to investigate reproductive issues and for the study of DOHaD. The

evidence to date supports the notion that more interventional studies can be performed in guinea pigs to develop and test the efficacy of potential therapeutic interventions following high-risk pregnancy. The guinea pig is also the ideal species to assess the multi- and trans-generational effects of both perinatal perturbation and any intervention. These translational data will inform clinical research and speed up the rate at which clinically important perinatal research can innovate and progress to safeguard the health of vulnerable pregnancies and vulnerable babies throughout their life course.

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## Additional information

### Competing interests

The authors have no competing interests.

### Author contributions (Fig. 7)

Conception or design of the work: J.L.M., K.J.B., J.R.T.D., A.L.D., K.L.G., K.L.K., S.G.M., T.R.H.R., L.P.T., M.J.B. Acquisition or analysis or interpretation of data for the work: J.L.M., K.J.B., J.R.T.D., R.M.D., K.L.G., C.G., E.A.H., J.J.H., B.K., K.L.K., B.J.K., S.G.M., H.K.P., T.R.H.R., B.S.R., A.S., L.P.T., M.J.B. Drafting the work or revising it critically for important intellectual content: J.L.M., K.J.B., J.R.T.D., A.L.D., R.M.D., K.L.G., C.G., E.A.H., J.J.H., B.K., K.L.K., B.J.K., S.G.M., H.K.P., T.R.H.R., B.S.R., A.S., L.P.T., M.J.B. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Figure 7. The authors