



THE IMPACT OF THE PERICONCEPTIONAL ENVIRONMENT

(IN VIVO AND EX VIVO)

ON FETO-PLACENTAL DEVELOPMENT IN THE SHEEP

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Abstract

A range of epidemiological, clinical and experimental studies have demonstrated that exposure of an embryo to a suboptimal environment *in vivo* or *ex vivo* during early embryo development is associated with altered development of cardiovascular, neuroendocrine and metabolic disorders in adult life. A number of perturbations during early embryo development result in developmental adaptations by the embryo to ensure immediate survival, whilst programming the embryo for altered fetal and placental development, resulting in the eventual onset of adult disease. It has been previously shown that maternal nutrient restriction during the periconceptual period results in a hyperactivation of the pituitary – adrenal axis and increased mean arterial blood pressure in twin but not singleton pregnancies.

It was therefore the first aim of this thesis to interrogate the impact of maternal undernutrition during the periconceptual period (defined as from at least 45 days prior until 7 days after conception) on fetal and placental development during early pregnancy at ~ day 55 of pregnancy, which coincides with the period of maximal placental growth. In Chapter 2, it has been demonstrated that there are important relationships between maternal weight gain during the periconceptual period and feto-placental growth during the first ~ 55 days of pregnancy and that periconceptual undernutrition has a differential effect on these relationships in singleton and twin pregnancies. In singleton pregnancies, periconceptual undernutrition disrupts the relationship between maternal weight

gain during the periconceptual period and utero-placental growth and in twin pregnancies, periconceptual undernutrition results in the emergence of an inverse relationship between maternal weight gain during early pregnancy and uteroplacental growth and in a dependence of fetal growth on placental growth. (Chapter 2)

In order to investigate the origins of the physiological adaptations that lead to the development of hyperactivation of the pituitary – adrenal axis and increased mean arterial blood pressure in late gestational fetuses after exposure as an embryo to periconceptual undernutrition, we investigated the development and steroidogenic capacity of the fetal adrenal gland and development of the fetal heart and kidney at ~ 55 days gestation (Chapter 3 and 4).

The relative weight of the fetal adrenal and adrenal IGF-1, IGF-1R, IGF-2, IGF-2R and CYP 17 mRNA expression were lower in twin compared to singleton fetuses. There was evidence that in control singletons, IGF-2R expression plays an important role in the regulation of adrenal growth and CYP 17 mRNA expression during early pregnancy. In control twins, however, whilst there was a significant positive relationship between adrenal CYP 17 and IGF-2 mRNA expression, adrenal weight was directly related to the level of adrenal IGF-1 mRNA expression. There was no effect of periconceptual undernutrition on the level of expression of any of the placental or adrenal genes in the study. In PCUN ewes, carrying singletons, however, there was a loss of the relationships between either adrenal IGF-2, IGF-2R and IGF-1 mRNA expression and adrenal growth and CYP 17 expression which were present in control singletons. Similarly in

ewes carrying twins, maternal undernutrition during the periconceptual period resulted in the loss of the relationships between adrenal growth and IGF-1 expression and between adrenal CYP 17 and IGF-2 expression which were present in control twin fetuses. Whilst there was no effect of fetal number on fetal heart growth at ~ d55 in twin fetuses, there was a direct relationship between relative fetal heart and adrenal weights, which was present in both the PCUN and control groups. There was also a significant inverse relationship between maternal weight at conception and relative fetal heart weight in PCUN twin, but not PCUN singleton or control fetuses (Chapter 3).

In control pregnancies maternal weight gain during the periconceptual period is inversely related to the relative weight of the fetal kidney at ~55d pregnancy. In this group, relative kidney weight was also directly related to renal IGF-1 mRNA expression. In control twins maternal weight gain was inversely related to fetal kidney weight and this effect was ablated when the effects of maternal cortisol was controlled for in the analysis. In the PCUN group, whilst there was an inverse relationship between maternal weight gain during the periconceptual period and relative kidney weight, it was not possible to separate the independent effects of maternal weight loss during the periconceptual period and the subsequent weight gain during the period of refeeding. Renal IGF-1 mRNA expression was higher and renal IGF-1R and 2R expression were lower in twin fetuses compared to singletons. After exposure to PCUN, renal IGF-1 expression was also higher than in control pregnancies independent of the fetal number (Chapter 4).

Superovulation, artificial insemination, embryo transfer and *in vitro* embryo culture are used in a range of assisted reproductive technologies, and it has been demonstrated that varying the composition of the culture media can result in a change in pre and postnatal development. Culture of sheep embryos in media containing serum is associated with fetal overgrowth which is phenotypic of the Large Offspring Syndrome. It is not known how the combination of superovulation, artificial insemination and embryo transfer alone impacts fetoplacental development in late gestation of the sheep. There have been no studies, however, examining the differential impact of superovulation, artificial insemination and embryo transfer with or without *in vitro* embryo culture in the absence or presence of human serum on fetoplacental development in singleton and twin pregnancies (Chapter 5).

I have therefore tested the hypothesis that superovulation, artificial insemination and embryo transfer with or without *in vitro* embryo culture in the presence or absence of human serum differentially alters the growth of the placenta, fetus and fetal organs during late gestation when compared to naturally conceived controls and that these effects are different in singleton and twin pregnancies. The fetal weight, CRL and abdominal circumference were significantly larger in IVCHS singleton fetuses. A novel finding in this study was lower fetal weights of twin fetuses in the ET and IVCNS groups compared to NM control twin fetuses. In addition, placental weights were lighter in twin fetuses in the ET, IVCNS and IVCHS treatment groups and this is partially due to a failure to initiate compensatory growth of placentomes in twin pregnancies (Chapter 5).

The results of this thesis therefore highlight the complex interactions between the periconceptual environment (*in vivo* or *ex vivo*) and embryo or fetal number on the programming fetal and placental development. Maternal undernutrition during the periconceptual period and superovulation, artificial insemination and embryo transfer with or without *in vitro* culture in the absence or presence of serum alters fetal development, and I have demonstrated that these changes in fetal growth can be explained by changes in placental growth trajectory. Furthermore, a novel finding of this study is that perturbations of the periconceptual environment affect feto-placental development differently in singleton and twin pregnancies.