Metabolic Phenotyping of Young Adults and Mice Born Through In Vitro Fertilization (IVF)

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This thesis is dedicated to my parents and my wife for their love.

My dream will not come true without you.

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Abstract

In vitro fertilisation (IVF) has been widely used to treat infertility since 1978. Worldwide, there are over 5 million children who have been born following assisted reproduction, mostly by IVF. However, the long term health implications of IVF are unknown. This thesis focuses on the metabolic risks of IVF in adult humans and mouse offspring.

A suboptimal in vivo environment during pregnancy, and the early postnatal period, predisposes offspring to chronic diseases later in life. Preimplantation embryos are also sensitive to adverse environmental insults in vivo or in vitro. Emerging evidence suggests that IVF children may be at an increased risk of developing metabolic and cardiovascular diseases. However, it is unclear if increased risk is related to the underlying genetics of the parents, environmental factors, or the treatment procedures per se which include both ovarian stimulation and embryo culture.

This is the first study to show that IVF adult humans were insulin resistant, by using gold standard assessment hyperinsulinemic-euglycemic clamp, compared to BMI and aged matched naturally conceived individuals after 3 days of a baseline energy balanced diet (30% fat), and that they tended to be more susceptible to the metabolic consequences of 3 days of high-fat overfeeding (+1250 kcal/day, 45% fat) as evidenced by a greater increase in systolic blood pressure.

To separate out potential genetic and environmental confounders as well as the effects of ovarian stimulation versus embryo culture, we developed an IVF mouse model using inbred C57BL/6J mice. Here, we examined glucose metabolism in adult offspring conceived by natural conception (NC), by ovarian stimulation alone (OS) or by IVF, and then fed a chow or high-fat diet (60% fat) for 8 weeks. Our data suggest it is the process of

IVF itself that contributes to impaired glucose metabolism in the adult mouse, which was more prominent in males. Moreover, we show that ovarian stimulation impairs fetal growth, and also results in glucose intolerance in offspring, which was unmasked by a high-fat diet in adult females. This study suggests that ovarian stimulation alone and IVF may program distinct metabolic effects in the offspring, but that high fat diet may be required to uncover these differences.

Our data shows that the preimplantation period is a critical stage for development and later adult health. The mechanisms underlying these differences are unclear, but may involve epigenetic modifications and/or changes in mitochondrial numbers and function. We initially examined whether altered DNA methylation and expression of key genes *PPARGC1A* and *IGF2* occurs in peripheral insulin sensitive tissues of morbidly obese individuals with or without type 2 diabetes. Our data showed that obese patients with and without type 2 diabetes displayed tissue specific DNA methylation of *PPARGC1A* and *IGF2*, highlighting the importance of measuring individual tissues in this response in humans and controlling for adiposity. Whether these alternations are evident in IVF conceived adults requires further study.

In conclusion, this study highlights an increased risk of developing type 2 diabetes and cardiovascular disease in IVF offspring later in life in an obesogenic environment.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Conference Proceedings

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List of Abbreviations

AKT: Protein Kinase B

ART: Assisted reproduction technology

ATP: Adenosine Triphosphate

AUC: Area under the curve

B2M: Beta-2 microglobulin

BMI: Body mass index

BWS: Beckwith-Wiedemann syndrome

CEBPα: CCAAT-enhancer-binding protein alpha

COC: Cumulus–oocyte complex

COX7A1: Cytochrome c oxidase (COX) subunit 7A, polypeptide 1

CpG: Cytosine-phosphate-guanine

Cpt1a: Carnitine palmitoyltransferase 1a

DEPC: Diethylpyrocarbonate

DMRs: Differentially methylated regions

DNA: Deoxyribonucleic acid

Dnmts: DNA methyltransferases

eCG: Equine chorionic gonadotropin

EDTA: Ethylenediaminetetraacetic acid disodium salt dihydrate

EGTA: Ethylene glycol-bis (2-aminoethylether) –N,N,N’,N’-tetraacetic acid

ER: Endoplasmic reticulum

ERK: Extracellular signal-regulated kinase

FAT/CD36: Fatty acid translocase/Cluster of Differentiation 36

FFAs: Free fatty acids

FFM: Fat free mass

FOXO1: Forkhead box O1

FTO: Fat mass and obesity associated

Gapdh: Glyceraldehyde-3-phosphate dehydrogenase

Gck: Glucokinase

GIR: Glucose infusion rate

GNASAS: Guanine Nucleotide Binding Protein Antisense RNA

GnRH: Gonadotrophin releasing hormone

G6pc: Glucose-6-phosphatase catalytic subunit

hCG: human chorionic gonadotropin

HDL: Higher high-density lipoprotein

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HFD: High fat diet

HOMA-IR: Homeostasis model of assessment - insulin resistance

Hprt: Hypoxanthine phosphoribosyltransferase

ICM: Inner cell mass

ICSI: Intracytoplasmic sperm injection

IGF: Insulin-like growth factor

IGF2: Insulin-like growth factor 2

IKK-β: IkB kinase-β

IL-6: Interleukin 6

IL10: Interleukin-10

INSIGF: Insulin – IGF

IR: Insulin resistance

IRR: Insulin receptor-related receptor

IRS: Insulin receptor substrate

i.p.: Intraperitoneal

IPGTT: Intraperitoneal glucose tolerance test

IPITT: Intraperitoneal insulin tolerance test

IVF: In vitro fertilization

i.v.: Intravenous

IVGTT: Intravenous glucose tolerance test

JNK-1: Jun kinase-1

MEG3: Maternally Expressed 3 (Non-Protein Coding)

MEST: Mesoderm-specific transcript

MtDNA: Mitochondrial DNA

mTOR: Mammalian target of rapamycin

NaF: Sodium fluoride

NaPPi: Sodium pyrophosphate tetrabasic decahydrate

NC: Natural conception

Ndufb5: NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5

NGAL: Neutrophil gelatinase-associated lipocalin

NNAT: Neuronatin

nPKCs: Novel protein kinase Cs

OS: Ovarian stimulation

OXPHOS: Oxidative phosphorylation

PAI-1: Plasminogen activator inhibitor-1

PBS: Phosphate buffered saline

PC-1: Plasma cell membrane glycoprotein-1

Pck1: Phosphoenolpyruvate carboxykinase 1, cytosolic

PCR: Polymerase chain reaction

PDK: Phosphoinositide-dependent kinase

PI3K: Phosphoinositol 3-kinase

PIP3: Phosphatidylinositol 3,4,5-trisphosphate

PKC: Protein kinase C

PTPase: Protein-tyrosine phosphatase

PPARγ: Peroxisome proliferator activated receptor gamma

PPARGC1A: Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; PGC1a; Pgc1a

Ppia: Cyclophilin-A

PVDF: Polyvinylidene difluoride

PVP: Polyvinylpyrrolidone

PWS: Prader-Willi Syndrome

RBP: Retinol-binding protein

RBP-4: Lipocalins retinol-binding protein 4

RNA: Ribonucleic acid

Rn18s: 18S ribosomal RNA

SAM: S-adenosylmethionine

SBTI: Soybean trypsin inhibitor

SCD1: Stearoyl-CoA desaturase-1

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SERPINF1: Pigment epithelium-derived factor; PEDF

Srebf1: Sterol regulatory element-binding transcription factor 1

SRS: Silver-Russell Syndrome

TBS: Tris-buffered saline

TBST: Tris-buffered saline with Tween 20

T2DM: Type 2 diabetes

TE: Trophectoderm

Tfam: Mitochondrial transcription factor A

TG: Triglycerides

TGFβ: Transforming growth factor β

TNF-α: Tumour neorosis factor α

UBE3A: E6-AP ubiquitin-protein ligase

WHO: World Health Organization