# Pre-implantation maternal uterine effects on embryo growth and development: An investigation using models of maternal constraint in sheep

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# Abstract

Prenatal development and growth are critical to survival of the fetus and neonate. Recent evidence suggests that a critical period for determining growth is the pre-implantation period of pregnancy during which differentiation, organogenesis and development of the embryo occur and the embryo is considerably vulnerable to uterine environmental factors. The objectives of the present study were to examine the effects of restrictive uterine environments on embryo development using two sheep models of maternal constraint: litter size and dam size, and to identify embryonic and maternally-driven mechanisms that regulate development of the peri-implantation sheep embryo.

Morphometric analysis (embryo length, width and heart bulge width) of the embryos in peri-implantation single and twin embryos was inconclusive; as was the transcriptomics analysis of whole embryos using RNA-seq to examine differential gene expression that may be responsible for differential regulation of growth.

In a dam size model, large-breed Suffolk embryos gestated in small-breed Cheviot ewes (constrained environment) were smaller than Suffolk embryos gestated in Suffolk ewes (control) at day 19 of pregnancy, confirming previous findings that maternal constraint is evident in early pregnancy when limitations of space are not of consequence. Progesterone administered in the post-ovulatory period, day 0 to 6, alleviates this apparent constraint such that Suffolk embryos gestated in Cheviot ewes that received progesterone are larger than those gestated in Cheviot ewes that did not. Further, differential gene expression analysis of maternal uterine tissues showed that at day 6 and day 19 endometrial genes that

encode for histotroph secretion and uterine receptivity are altered by post-ovulatory progesterone administration. Timing of administration of progesterone is critical not only to embryo growth but also to embryo survival. There were lower pregnancy rates in the ewes that received progesterone from day 0 than those that received progesterone from day 2.

The results of this thesis indicate that progesterone exerts its effects by regulation of genes that encode for uterine structural and secretory activity to advance the uterus. This likely forces the asynchronous embryo to accelerate its growth in order to adapt to its environment. These findings contribute to the knowledge of the regulatory mechanisms controlling early embryo growth and present a platform within the livestock industry and human reproductive technology practice to manipulate embryo growth to improve survival of offspring.

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# List of Abbreviations

ACTB = Beta actin

AI = Artificial insemination

BNC = Binucleate cells

Bp = Base pair

C = Cheviot

cDNA = Complementary Deoxyribonucleic acid

CIDR = Controlled intravaginal progesterone drug releasing device

CL/s = corpus luteum/corpora lutea

Con1E1CL = Control singleton bearing ewe (single CL), no embryo transfer

Con 2E2CL = Control twin bearing ewe (two CLs), no embryo transfer

COX2 = Cyclooxygenase 2

CP4 = Cheviot ewe that receive progesterone from day 0 to day 6 of pregnancy

CnP4 = Cheviot ewe that did not receive progesterone from day 0 to day 6 of pregnancy

Ct = Quantification cycle

CTSL = Cathepsin L

CV% = coefficient of variance

DEG = differentially expressed gene

DGAT2 = diacylglycerol-O-acyltransferase

DKK4 = Dickkopf WNT signalling pathway inhibitor 4

EL = embryo length: distance from the medial aspect of the head to the tip of the embryonic tail, following the outer curvature of the embryo

ET = Embryo transfer

EGF = Epidermal growth factor

ER/ESR1 = Estrogen receptors

EW = Embryo width: distance between the two widest points of the embryo with the line passing just below the heart bulge

FDR = False discovery rate

FGF1 = Fibroblast growth factor 1

FGF2 = Fibroblast growth factor 2

FGF7 = Fibroblast growth factor 7

FGF10 = Fibroblast growth factor 10

GAPDH = Glyceralydehyde-3-phosphate dehydrogenase

GE = Glandular epithelium

GH = placental growth hormone

HB = Heart bulge width: distance between the two widest points of the heart bulge with the line passing through the midsection of the heart bulge

HGF = Hepatocyte growth factor

HPRT = Hypoxanthine phosphoribosyltransferase 1

IGFs = Insulin like growth factors

IGF1 = Insulin like growth factor 1

IGF2 = Insulin like growth factor 2

IGF1R = Insulin like growth factor 1 receptor

INFτ = Interferon tau

INFAR = Type 1 interferon receptors

IRF2 = Interferon regulatory factor 2

ISG17 = Interferon stimulated gene 17

IUGR = Intrauterine growth restriction

IV = Intravenous

LAPTM5 = Lysosomal-associated protein transmembrane 5

LE = Endometrial luminal epithelium

*LGALS3* = Lectin galactoside-binding, soluble 3

LGALS15 = Endometrial galectin 15/ Lectin galactoside-binding soluble 15

LOC101103603 = Pregnancy associated glycoprotein-4 like

LOC101117738 = Pregnancy associated glycoprotein-1 like

LRRC32 = Leucine rich repeat containing 32

MET = C-met proto-oncogene

mRNA = Messenger RNA

MSTN = Myostatin

MUC1 = Mucin glycoprotein 1

NFW = Nuclease free water

OXTR = Oxytocin receptor

PBS = Phosphate buffered saline

PCR = Polymerase chain reaction

$PGF_{2\alpha}$ = Prostaglandin $F_{2\alpha}$

PL = Placental lactogen

PGR = Progesterone receptors

PTGS2 = Prostaglandin endoperoxide synthase 2

P4 = Progesterone

qPCR = Quantitative real time PCR

RIN = RNA Integrity number

RNA = Ribonucleic acid

RPL19 = Ribosomal protein L 19

RSAD2 = Radical S-adenosyl methionine domain containing 2

RT = Reverse transcriptase

RT-qPCR = Reverse transcriptase quantitative PCR

S = Suffolk

SinCP4 = Suffolk embryo that was gestated in a Cheviot ewe that receive progesterone from day 0 to day 6 of pregnancy

SinCP4$^{0-3}$ = Suffolk embryo that was gestated in a Cheviot ewe that receive progesterone from day 0 to day 3 of pregnancy

SinCP4$^{0-6}$ = Suffolk embryo that was gestated in a Cheviot ewe that receive progesterone from day 0 to day 6 of pregnancy

SinCP4$^{2-4}$ = Suffolk embryo that was gestated in a Cheviot ewe that receive progesterone from day 2 to day 4 of pregnancy

SinCP4$^{3-6}$ = Suffolk embryo that was gestated in a Cheviot ewe that receive progesterone from day 3 to day 6 of pregnancy

SinCnP4 = Suffolk embryo that was gestated in a Cheviot ewe that did not receive progesterone from day 0 to day 6 of pregnancy

SinSP4 = Suffolk embryo that was gestated in a Suffolk ewe that received progesterone from day 0 to day 6 of pregnancy

SinSnP4 = Suffolk embryo that was gestated in a Suffolk ewe that did not receive progesterone from day 0 to day 6 of pregnancy

SERPIN = Uterine serine proteinase inhibitor/ Uterine milk proteins

sGE = Superficial glandular epithelium

SPP1 = Secreted phosphoprotein 1/osteopontin

SP4 = Suffolk ewe that received progesterone from day 0 to day 6 of pregnancy

SnP4 = Suffolk ewe that did not receive progesterone from day 0 to day 6 of pregnancy

TGF = Transforming growth factor

TKDP = Trophoblast Kunitz domain protein-1

*TP1* = Trophoblast protein 1

UGKO = Uterine gland knock out

UTMP = Uterine Milk Proteins

YWHAZ = Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta.

1E1CL = Singleton embryos (harvested from a ewe with a single CL) transferred into a ewe that was also identified as having a single CL, and a single embryo that was removed

1E2CL = Singleton embryos (harvested from a ewe with a single CL) transferred into a ewe that was identified as having two CLs, and twin embryos that were removed

2E1CL = Twin embryos (harvested from a ewe with two CLs) transferred into a ewe that was identified as having a single CL, and single embryo that was removed

2E2CL = Twin embryos (harvested from a ewe with two CLs) transferred into a ewe that was also identified as having two CLs, and twin embryos that were removed