The Role of Follistatins in Parturition in Women

By

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Declaration

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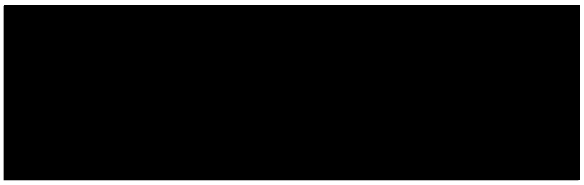
I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.



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Publications arising from this thesis

Papers

1. K. Rae , K. Hollebone, V. Chetty, D. Clausen, J. McFarlane, *Follistatin serum concentrations during full-term labour in women; significant differences between spontaneous and induced labour*, (Accepted by Reproduction, 19th June, 2007)

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3. K. Rae , K. Hollebone, V. Chetty, D. Clausen, J. McFarlane, *Maternal serum follistatin is affected by fetal gender,* Unsure of target journal

Conference Abstracts

1. Rae K., Xia, Y., O'Shea, T., McFarlane, J, 2003, *Follistatin immunoreactive profiles across parturition in ewes using different assays*, Endocrine Society of Australia Annual Conference, Melbourne, Australia.

2. Rae, K., Hollebone, K., Clausen, D., Chetty, V., McFarlane, J., 2004, *A Cross-Sectional Study of Follistatin During Labour in Women*, Endocrine Society of Australia Annual Conference, Sydney, Australia.

3. Rae, K., Hollebone, K., Clausen, D., Chetty, V., McFarlane, J., 2004, *Maternal Serum Follistatin Concentrations are Influenced by Foetal Sex*, Endocrine Society of Australia Annual Conference, Sydney, Australia.

4. Rae, K., Hollebone, K., Clausen, D., Chetty, V., McFarlane, J., 2004, *Follistatin Changes Significantly in the Spontaneous Labouring patient*, Endocrine Society of America Annual General Meeting, New Orleans, United States of America

5. Rae, K., Hollebone, K., Meng, L., Clausen, D., McFarlane, J., 2005, *A Differential Pattern of Follistatin Expression in the Placenta between Spontaneous, Induced and Non-Labouring Patient Groups*, Endocrine Society of Australia Annual Conference, Perth, Australia.

6. Rae, K., Hollebone, K., Meng, L., Clausen, D., McFarlane, J., 2005, *Immunohistochemistry of Follistatin shows a Differential Expression Correlating with Differing Labour Groups in Women*, American Society for Reproductive Medicine Annual Scientific Meeting, Montreal, Quebec, Canada

Glossary of abbreviations

aa or AA	amino acids
Act/FS	activin/follistatin complex
ACTH	adrenocorticotrophin hormone
ActRII	activin type II receptor (ligand binding)
ActRI	activin type I receptor (signal transducing)
ALK	activin receptor-like kinase
ANTE	antenatal
ANOVA	analysis of variance
APP	acute phase proteins
ARIP	activin receptor interacting proteins
BCIP	5-bromo-4-chloro-3-indoyl phosphate
11βHSD-2	11β hydroxysteroid dehydrogenase 2
BMP	bone morphogenetic protein
BMP/FS	bone morphogenetic protein/ follistatin complex
BMP-R	bone morphogenetic protein receptor
BSA	bovine serum albumin
cACTH	chorionic adrenocorticotrophin hormone
CAM	cell adhesion molecules
cAMP	cyclic adenosine monophosphate
CRH	corticotrophin releasing hormone
CRP	C reactive protein
CSF	cerebrospinal fluid
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
EDTA	ethylene diamine tetra-acetic acid
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
Fc	group of immunoglobulin binding receptors
FS	follistatin

FS-	follistatin knockout
FS+	follistatin over-expresser
FSH	follicle stimulating hormone
FLRG	follistatin related gene
FSRP	follistatin related protein
FSTL	follistatin like protein
FS288/Act	follistatin isoform 288/ activin complex
FS315/Act	follistatin isoform 315/ activin complex
FS288	follistatin isoform 288
FS303	follistatin isoform 303
FS315	follistatin isoform 315
GASP	growth and differentiation factor associated serum protein
G-CSF	granulocyte colony stimulating factor
GDF	growth differentiating factor
GDNF	glial cell line derived neutrophic factor
GGT	gammaglutamyltransferase
GHRH	growth hormone releasing hormone
GH-V	growth hormone variant; chorionic somatomammotropin
GnRH	gonadotropin releasing hormone
GRE	glucocorticoid response elements
HB-EGF	heparan binding epidermal growth factor
HBS	heparan binding sequence
hCG	human chorionic growth factor
HDL	high density lipoprotein
hPL	human placental lactogen
HepG2 cells	perpetual human liver epithelial cell line
IFNα	interferon α
IgG	immunoglobulin G
IL	interleukin
IND	induced onset of labour
IVF	*in vitro* fertilization

IV	intravenous
kb	kilobases
Kd	dissociation constant
kDa	kilodalton
KGF	keratinocyte growth factor
LAB1	early labour (< 3cm vaginal dilation)
LAB2	late labour (> 3cm vaginal dilation)
LH	lutenizing hormone
LPS	lipopolysaccharide
LSCS	lower segment caesarian section (nil onset of labour)
MIC-1	macrophage inhibitory cytokine-1
MIS	maturation inducing steroid
MMP	matrix metalloproteinases
mRNA	messenger RNA
MSAFP	maternal serum α fetoprotein
MyD88	myeloid differentiation primary response
NFκB	nuclear factor κ B
NK	natural killer cells
NPP	p-nitrophenyl phosphate, disodium salt hexahydrate
OP	osteogenic protein, SPARC and BM-40
PCOS	polycystic ovarian syndrome
PCR	polymerase chain reaction
PBS	phosphate buffered saline
PG	prostaglandins
PG_{E2}	prostaglandin E2
$PG_{F\alpha}$	prostaglandin Fα
POST1	early post- partum (< 3hours from delivery)
POST2	late post-partum (>3 hours from delivery)
PRL	prolactin
PTH-rP	parathyroid hormone related protein
RBC	red blood cells

RNA	ribonucleic acid
RT	reverse transcription
SARA	Smad anchor for receptor activation
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate- polyacrylamide gel electrophoresis
SEM	standard error margin
SNK	Student-Newman-Keuls post test
SPON	spontaneous onset of labour
TAE	tris acetate EDTA buffer
TEMED	N,N,N',N'-tetramethylenediamine
TGFβ	transforming growth factor β
TGFα	transforming growth factor α
TIMP	tissue inhibitor of metalloproteinases
TLR	toll-like receptor
TNFα	tumor necrosing factor α
TNFγ	tumor necrosing factor γ
TRH	thyrotropin releasing hormone
TUN	trophouteronectin
UV	ultraviolet
uNK	uterine natural killer cells
VEC	vascular endothelial cells

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Abstract

Follistatin is monomeric protein that binds activin with high affinity and modulates its bioactivity. It exists in a number of different isoforms with FS288 and FS315 being the major forms. Recent work by Sidis *et al.* (2006) and Glister *et al.* (2006) indicate follistatin isoforms may have distinctly different roles and be differentially regulated in aspects of physiology (Glister *et al.*, 2006; Sidis *et al.*, 2006).

Follistatin has been widely investigated for its role in both female reproduction and inflammation. The onset of parturition in women shows many similarities to inflammatory events and the trigger for it remains unknown. Follistatin shows a wide distribution throughout the mammalian system, and in pregnancy it is found in fetal tissues, placenta and associated membranes. However the role of follistatin in onset of parturition remains unclear with the multitude of follistatin isoforms creating great difficulty in the development of specific assays and interpretation of results.

In this study on women, follistatin isoform expression in the placenta, follistatin in maternal and fetal circulation, and follistatin localization within the placenta were investigated and compared using women who have undergone spontaneous onset of labour, induced onset of labour and those with no onset of labour. Using both heparin binding affinity columns and Con A chromatography placental follistatin isoforms have been investigated to determine differences due to labour onset. Follistatin in fetal and maternal circulation was also investigated with regard to fetal gender, parity and length of labour.

Using primers designed specifically for FS288 and FS315 isoforms, reverse transcription polymerase chain studies have shown that women who have a spontaneous onset of

labour, express mRNA for both FS288 and FS315 in placenta. Those who undergo an induction or have a planned caesarean delivery with no onset of labour only show the FS315 isoform. It appears that FS315 isoform is the predominant mRNA in all patient groups when compared to the housekeeper β-actin and FS288. These results suggest that FS288 is a potential trigger for the onset of labour in women. Western Blotting analysis of placental homogenate studies using antiserum raised against a peptide (FS121-133) corresponding to amino acids 121-133 of follistatin, and found in all follistatin isoforms, show strongest recognition of larger molecular weight proteins 65-82kDa in placenta. These larger molecular weight proteins have been previously reported however, they remain uncharacterized. This study has shown that they are intrinsically linked with parturition in women and exhibit a variety of heparin binding and glycosylated forms. As the antibody used is specific to follistatin domain 1 we suggest that this protein is a new member of the follistatin family and critical for labour onset.

Concentrations of total circulating follistatin in the antenatal, parturition and postpartum period, measured by an assay using a human recombinant follistatin (FS288) as both standard and tracer and an antiserum raised against purified 35kDa bovine follistatin, are significantly higher in women that undergo spontaneous onset of labour and elevated in those undergoing induction of labour suggesting that follistatin is crucial to the labouring process. Additionally, assays for activin A show no correlations with follistatin, with greater activin concentrations seen in induced patients, suggesting the actions of follistatin are independent of those of activin. Studies using inflammatory cytokines, and acute phase proteins show that TNFα has no role in either onset of labour or postpartum inflammation, whilst C-reactive protein is up-regulated between 3-12 hours following delivery. These results indicate that the acute phase response is

important in late postpartum healing whilst the role of follistatin and activin in the postpartum phase is still unclear. However, follistatin concentrations showed no correlations with the acute phase response.

Fetal gender studies indicate that follistatin is higher in the male fetal circulation than females and higher in maternal serum of those carrying males. However it appears that the increased concentrations seen in male carrying mothers is not due to secretion from the male fetus but due to the maternal response. The length of labour also appears to influence follistatin concentration in both the fetus and the mother however increased patient numbers are needed to truly elucidate differences.

Immunohistochemistry studies using antiserum raised against a peptide (FS121-133) corresponding to amino acids 121-133 of follistatin, and found in all follistatin isoforms, showed that only spontaneous labour patients have follistatin protein localised to the syncytiotrophoblasts of the placenta. Both spontaneous and induced patients show follistatin in the vascular endothelial cells of the placenta whilst caesarean patients show none. Interestingly spontaneous and caesarean patients show follistatin staining in the maternal decidual cells whilst induced patients only have faint traces of the protein there. We propose that a lack of follistatin in the maternal decidua is the reason that some women fail to advance into parturition, and that through the natural onset of labour follistatin protein becomes expressed in the syncytiotrophoblast cells of the chorionic villi.

Taken together, the present studies have shown that follistatin isoforms and new members of the follistatin family are likely to play an important role in the onset of labour in women and may subsequently alter with fetal gender, labour length and parity.