

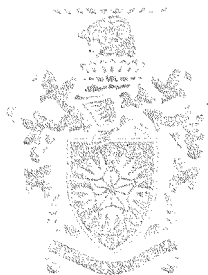
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THE PATTERN AND REGULATION OF MAMMARY GLAND DEVELOPMENT DURING FETAL LIFE IN THE SHEEP

A thesis presented in partial fulfilment of the requirements
for the degree of Doctor of Philosophy in Animal Science
at Massey University, Palmerston North, New Zealand

Catriona Margaret Christie Jenkinson

2003



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This is to certify that the research carried out in the Doctoral Thesis entitled
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GENERAL ABSTRACT

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The production of colostrum and milk in sufficient amounts is essential for the survival of the neonate. Although there is limited data to indicate that the extent of fetal mammary development is essential to subsequent milk production, the secretory epithelial cells that proliferate during pregnancy do so on the epithelial ducts that have developed during prenatal life. Thus any reduction in duct development may ultimately impact on secretory cell mass and hence the capacity of the adult gland to produce milk.

A series of studies were carried out to establish patterns of fetal mammary gland development between male and female sheep and to identify factors that may be involved in the regulation/mediation of growth and differentiation, and that may contribute to the sexual dimorphism of the gland. In addition, mammary gland development was measured in fetuses from ewes in which the maternal environment was altered by hormones, nutrition or pre-lamb shearing.

The sequence of events in the development of the mammary gland of the fetal sheep was similar to that described for cattle. Sexual dimorphism in the ovine gland became pronounced during the formation of secondary ducts and was especially evident during the development of the fat pad where adipose tissue was far less abundant from the outset in the male. In terms of epithelial development, total duct area was similar in males and females up until day 120 of fetal age. Between days 120 and 140 of fetal age, total duct area doubled in females while the interval between day 140 and three weeks of postnatal age witnessed a four- to five-fold increase in the size of the duct system. Conversely, the male gland failed to progress beyond that observed at day 120. The sex differences observed in the histomorphogenesis of the gland were reflected in a relative growth analysis of mammary development. The growth of the mammary gland in the female followed the general development of the fetus, while in the male, mammary growth exhibited negative allometry from day 80 to 140 of fetal age.

Further experiments investigated factors that may be involved in the regulation of epithelial and mesenchymal growth within the fetal mammary gland. Receptors for androgen and oestrogen were localised in the mammary epithelial and mesenchymal cells of both sexes. An association between the localisation of androgen receptors (AR) and the divergence in the pattern of mammary development between males and females suggested the involvement of androgens in the sexual dimorphism of the gland. In support of this suggestion was the observation of a similar pattern of mammary development and AR immunoreactivity in the mammary glands of female fetuses whose dams were injected with testosterone during early gestation. Insulin-like growth factor-I receptor (IGF-IR) followed a similar pattern of immunoreactivity to AR in the mammary

tissue of the male indicating that the suppression of mammary growth may be mediated by IGFs. IGF-IR immunoreactivity tended to increase in both the epithelial and mesenchymal cells of the female mammary gland as gestation progressed. An abundance of IGF-IR in the developing fat pad of the female gland suggested a role for locally derived IGFs in stimulating adipose tissue growth and hence, the continued proliferation and morphogenesis of epithelial cells.

The final study demonstrated that a low plane of maternal nutrition throughout pregnancy was detrimental to development of the fetal mammary gland and hence, its future capacity to produce milk. In terms of total duct area, fetal mammary growth was more than two-fold greater in fetuses whose dams were exposed to a high plane of nutrition throughout pregnancy than in those fetuses whose dams remained at maintenance. This substantial difference in the amount of epithelial tissue present occurred without any significant effect on fetal or gland weights. Moreover, the increase in total duct area associated with a higher plane of maternal nutrition closely mirrored the increase in the intensity of IGF-IR immunostaining in the epithelial cells.

In conclusion, these results provide indirect evidence that inhibition of mammary gland growth in the fetal male sheep is dependent on its exposure to testosterone and may involve mediation by IGF-I. Oestrogens may act directly or indirectly, mediated by oestrogen-induced IGFs from the mesenchymal cells, to stimulate epithelial cell differentiation and proliferation in the mammary gland of the fetal female sheep. Furthermore, strong evidence indicates that the ewe is able to influence mammary development in her female offspring *in utero*, which may eventually affect their potential to produce milk.

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LIST OF ABBREVIATIONS

AR	androgen receptor
BP	binding protein
CA	cyproterone acetate
cm	centimetre
CP	crude protein
CRL	crown-rump length
CT	computer-assisted tomography
DDE	dichlorodiphenyl dichloroethane
DDT	dichlorodiphenyl trichloroethane
DE	digestible energy
DES	diethylstilbestrol
DM	dry matter
DNA	deoxyribonucleic acid
3-D	three-dimensional
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
FGF	fibroblastic growth factor
FGFR	fibroblastic growth factor receptor
GH	growth hormone
GHR	growth hormone receptor
h	hour(s)
IGFBP	insulin-like growth factor binding protein
IGF	insulin-like growth factor
IGF-IR	insulin-like growth factor-I receptor
iu	international unit
kg	kilogram(s)
KGF	keratinocyte growth factor
kPa	kilopascals

LEF-I	lymphoid enhancer factor-1
Mcal	megacalorie
ME	metabolisable energy
mg	milligram(s)
MJ	megajoules
ml	millilitre(s)
mm	millimetre(s)
mRNA	messenger ribonucleic acid
OR α	oestrogen receptor alpha
OR β	oestrogen receptor beta
ORKO	oestrogen receptor knockout mouse
pi	post injection
PTHrP	parathyroid hormone-related protein
PTHR1	type 1 parathyroid hormone receptor
PUF	protected polyunsaturated fats
Tfm	testicular feminisation
TGF	transforming growth factor
μ g	micrograms
μ l	microlitres