Ewe nutrition influences fetal mammary gland biochemical indices and the abundance of mTOR pathway proteins

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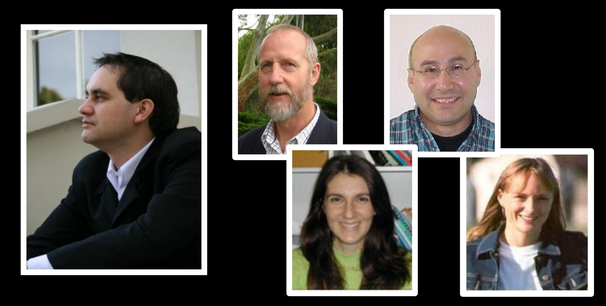


Introduction

Maternal nutrition has a significant effect upon the developing fetal mammary gland and future lactation potential. A pathway known to be regulated by nutrition is mTOR (mechanistic target of rapamycin), which integrates multiple environmental and metabolic signals to control cellular proliferation, differentiation and function. To date, there is no published literature describing the mTOR pathways potential involvement in fetal mammary gland development.

Objective

The objective of this study was to determine if increased fetal mammary gland size is associated with changes in biochemical indices and mTOR pathway signaling.

Trial design

Twin-bearing ewes

Nutrition	Day-100	Day-140
Ad-libitum	*n=16*	*n=12*
Maintenance (maintain conceptus-free weight)	*n=7*	*n=8*

Harvest fetal mammary glands

Biochemical indices

Method and Results

Total DNA, RNA and protein were extracted from fetal mammary glands and quantified. Total RNA, DNA and protein, RNA to DNA, protein to DNA and protein to RNA ratios were analysed for differences between treatment groups using the T-TEST procedure in SAS. Differences between means were considered significant at $*P \leq 0.05$, trends at $\dagger P \leq 0.15$.

Day-100 fetal mammary glands

	Ad libitum	Maintenance	P-value
No of Ewes (No of Samples)	10 (16)	5 (7)	
Mammary weight (g)	3.98±0.12	4.53±0.20	P=0.028
Total DNA per gland (mg)	0.83±0.03	0.75±0.04	P=0.100†
Total RNA per gland (mg)	0.57±0.02	0.53±0.03	P=0.340
Total Protein per gland (mg)	1.99±0.03	1.97±0.05	P=0.712
RNA : DNA	0.26±0.03	0.22±0.05	P=0.484
Protein : RNA	1.42±0.03	1.44±0.05	P=0.769
Protein : DNA	1.16±0.04	1.22±0.06	P=0.454

Increased mammary gland weight and a tendency for increased total DNA (cell number) was observed in day-100 fetuses from maintenance fed ewes compared to *ad libitum* fed.

Day-140 fetal mammary glands

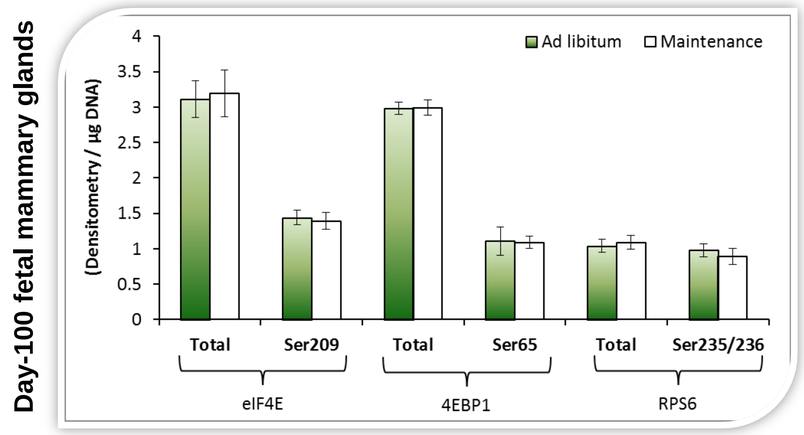
	Ad libitum	Maintenance	P-value
Ewe No (Sample Number)	9 (12)	7 (8)	
Mammary weight (g)	12.48±0.47	10.31±0.61	P=0.014
Total DNA per gland (mg)	7.24±0.59	4.82±0.77	P=0.026
Total RNA per gland (mg)	7.41±0.61	8.02±0.83	P=0.596
Total Protein per gland (mg)	188.32±19.97	151.26±26.98	P=0.326
RNA : DNA	1.13±0.15	1.75±0.0.20	P=0.025
Protein : RNA	26.14±2.76	19.95±3.56	P=0.191
Protein : DNA	27.71±3.93	35.09±5.08	P=0.270

Increased mammary gland weight was observed in day-140 fetuses harvested from *ad libitum* compared to maintenance-fed ewes. This was associated with increased total DNA, but reduced RNA : DNA ratio (protein synthesis capacity).

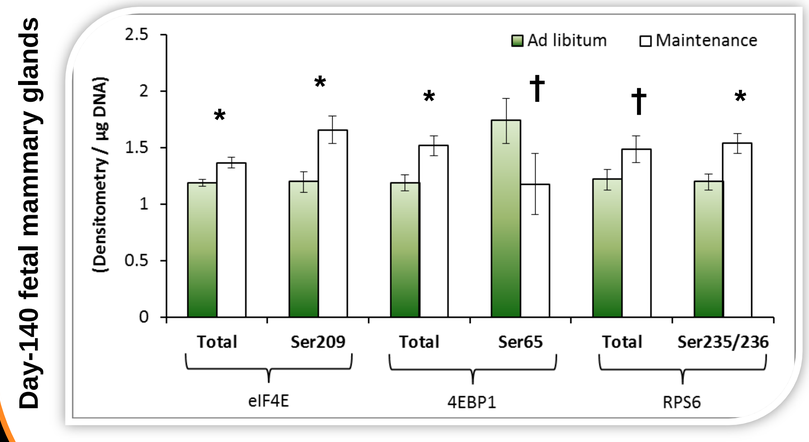
Western Blotting – mTOR Pathway

Method and Results

Protein was separated by SDS-PAGE, transferred by iBlot to PVDF membranes and probed with primary and secondary antibodies (Cell Signalling, Boston, MA, USA). At least 3 films were scanned, individual signal intensity was determined using ImageJ software. Individual signal intensities were then calculated per unit of DNA to express relative abundance per cell and then each value normalised against the sum of normalised values. Differences between treatment groups were analysed using the TTEST procedure in SAS. Differences between means were considered significant at $*P \leq 0.05$, trends at $\dagger P \leq 0.15$.



Ewe nutrition had no effect on the abundance of total and phosphorylated mTOR signaling proteins measured in day-100 fetal mammary glands.



Fetal mammary glands collected from maintenance fed ewes had increased abundance of total eIF4E, eIF4E^{Ser209}, 4EBP1, RPS6$^{Ser235/236}$ ($*P \leq 0.05$), and RPS6 ($\dagger P \leq 0.15$) compared to *ad libitum* fed. Total total 4EBP1^{Ser65} was decreased in fetal mammary glands collected from maintenance compared to *ad libitum* fed ewes

Conclusions

- At 100 days gestation, fetal mammary gland from maintenance fed ewes were larger and tended to have greater cell number compared to lambs born to ad libitum fed ewes. However, this effect of maternal nutrition was reversed by 140 days gestation indicating more rapid mammary growth in fetuses from ad-libitum compared to maintenance ewes in the last trimester.
- Despite reduced cell number and corresponding smaller mammary glands at 140 days of gestation, fetuses from maintenance fed ewes have mammary glands that are “developmentally” more active. This effect may help to explain why ewe lambs born to maintenance fed ewes, from the same trial (published van der Linden DS et al. 2009. J. Anim. Sci87(12):3944-54), have greater milk yields, lactose percentage and accumulated lactose yields compared with ad libitum fed ewes.

Acknowledgements
The authors gratefully acknowledge funding from the AgResearch Capability Fund (Maori PhD fellowship for Q Sciascia) and National Centre for Growth and Development (NRCGD). The animal trial, from which the tissues used in this experiment were derived, was conducted by Massey University with funding from the NRCGD.