Investigating effects of calcium and vitamin D metabolism on reproduction

Ian J Lean
Helen M Golder
Scibus, Camden, Australia
Rachael Rodney
Australian National University
Jose P Santos
Achilles Viera Neto
University of Florida

OBSERVATIONAL STUDIES

Clinical hypocalcemia: health and reproduction

- Milk fever cows are :
  - 8 times more likely to get mastitis
  - 3 times more likely to suffer from dystocia
  - 2 – 3 times more likely to suffer from retained placenta
  - 2 – 4 times more likely to develop DA (Houe et al., 2001)

- **Likely to have a longer calving to conception interval (12 days longer) (Borsberry and Dobson, 1989)**

- **Likely to require more services per conception (40 – 50% more services per conception)**

Subclinical: Time to Pregnancy (Caixeta et al., 2017)

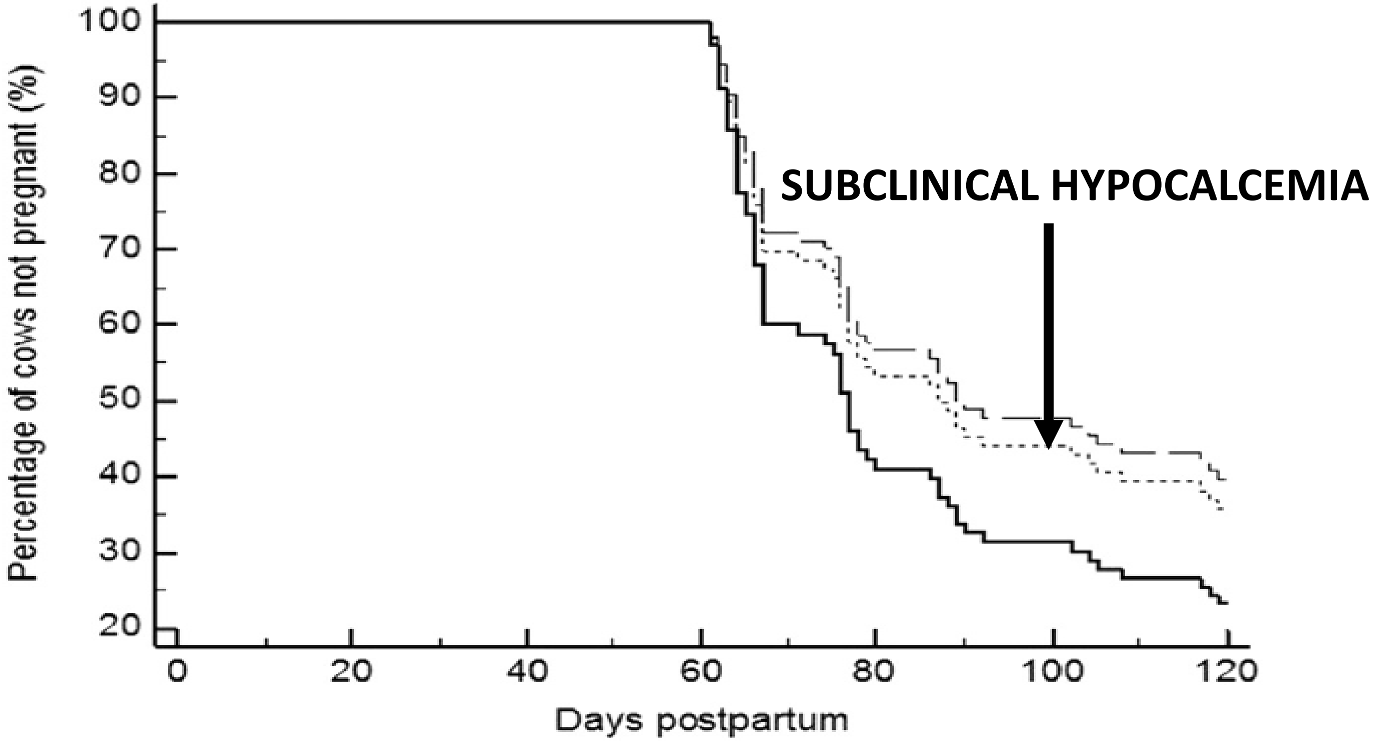
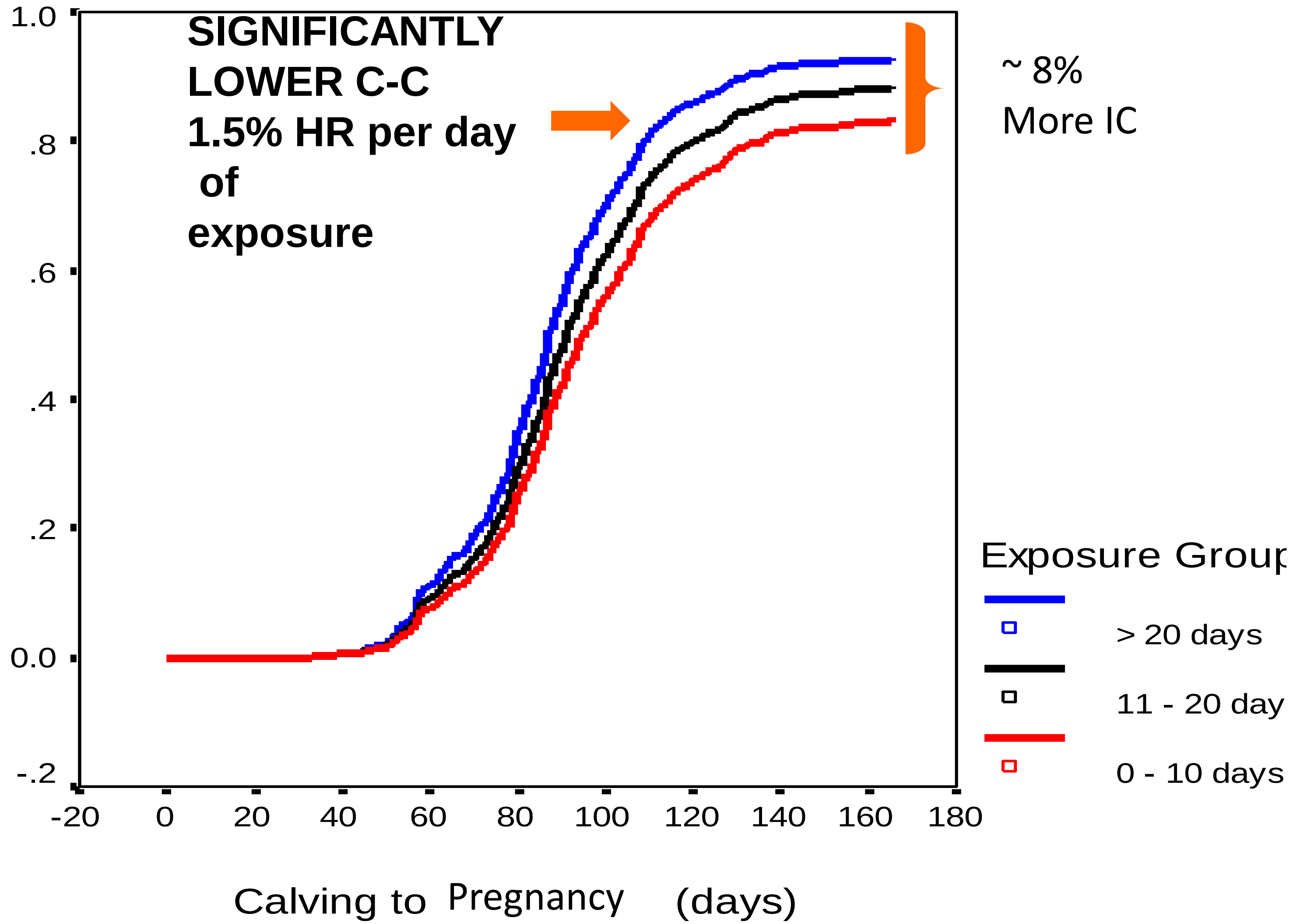
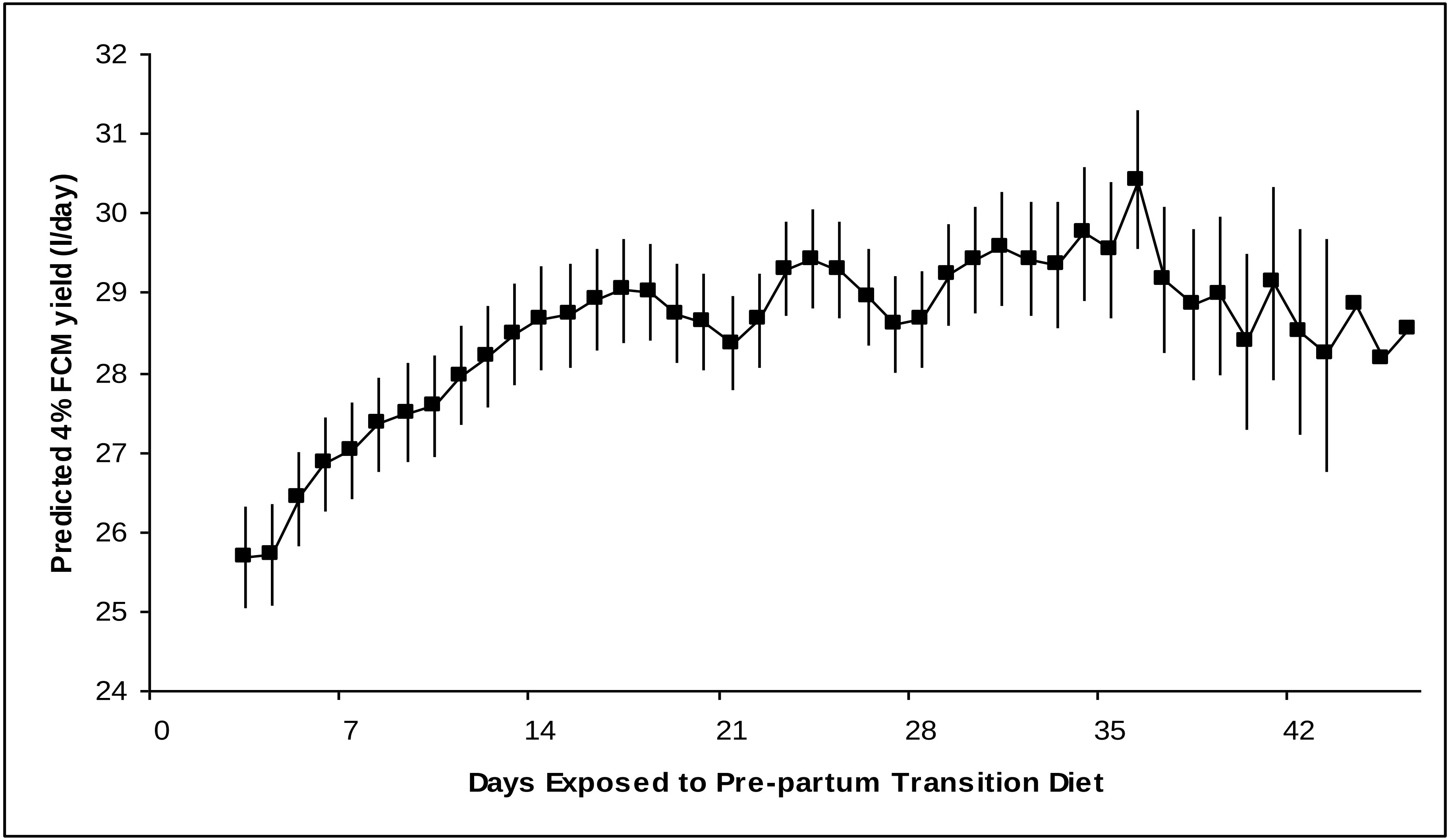


Fig. 3. Cox Proportional-Hazard curves for time to pregnancy in the first 120 DIM for eucalcemic animals (eucalcemia; n = 19; solid line), subclinical hypocalcemia (SCH; n = 45; dashed line) or chronic subclinical hypocalcemia (cSCH; n = 33; dotted line). Compared to the eucalcemic group the hazard ratio for subclinical hypocalcemic cows was 0.63 (95% CI = 0.33–1.23; $P = 0.2$) and 0.71 for chronic SCH (95% CI = 0.36–1.39; $P = 0.3$).

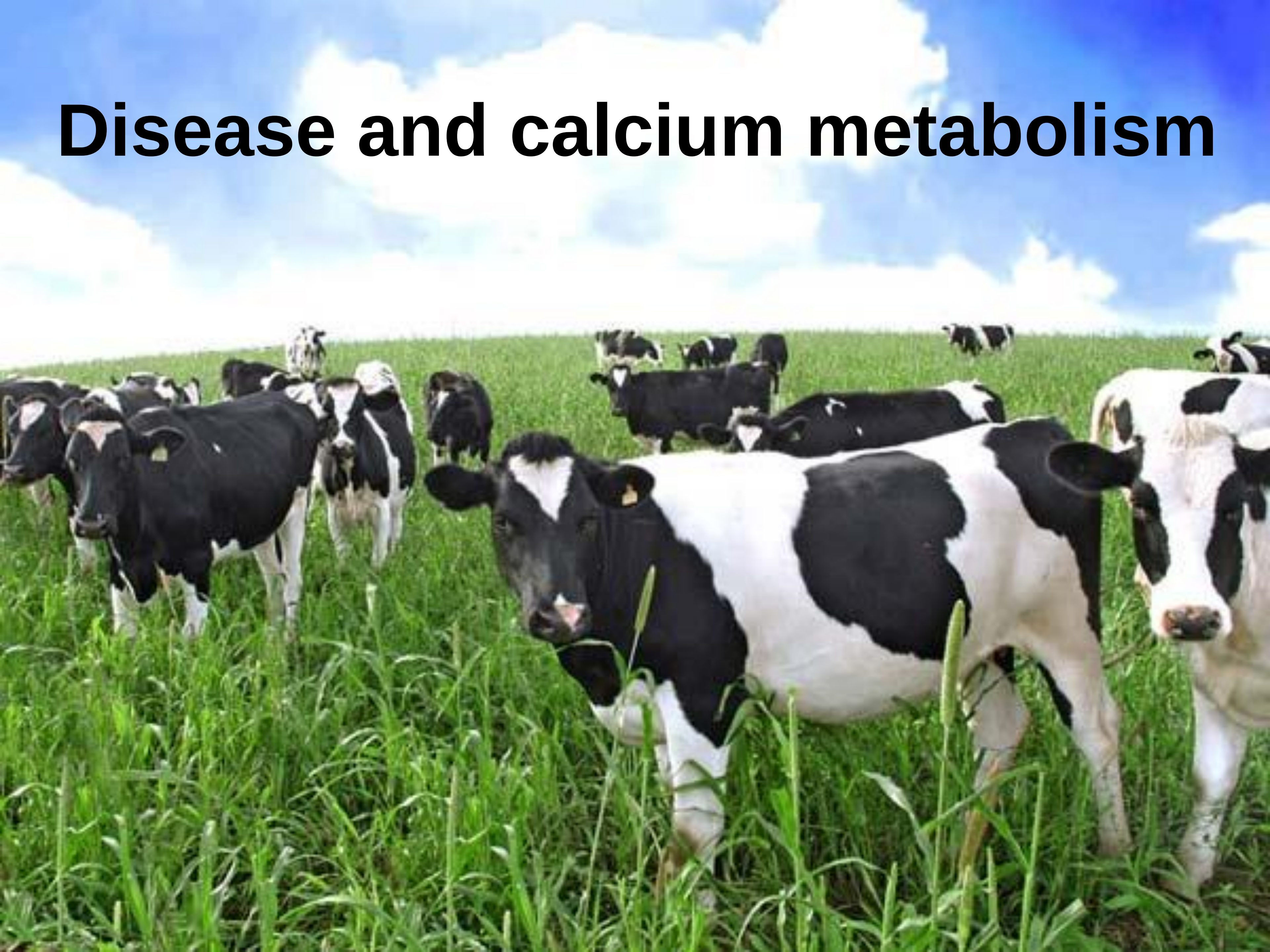
Cohort – Exposure to Transition diet: Time to Pregnancy (DeGaris et al., 2010 Aust Vet J)



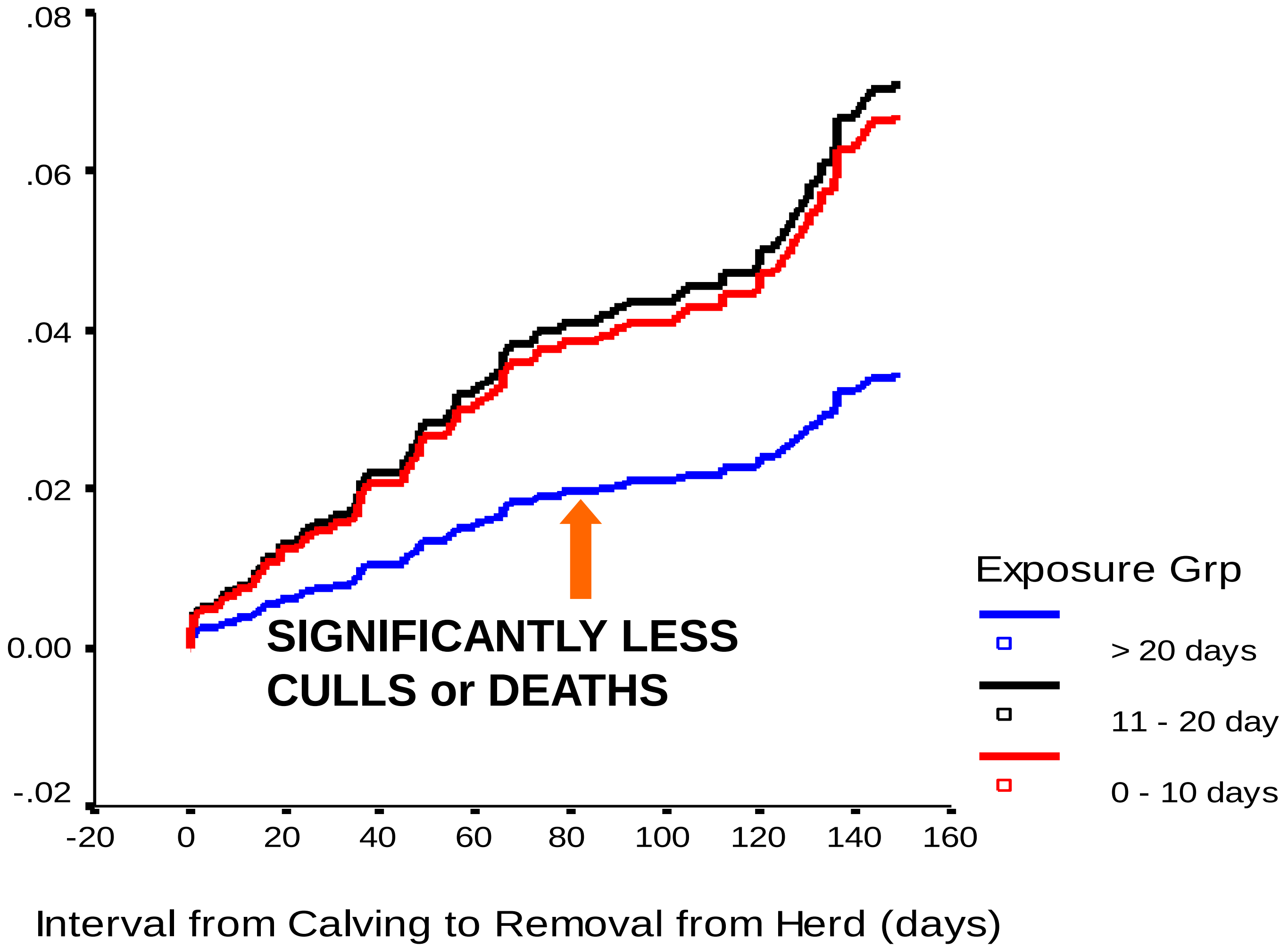
Increased exposure also increased energy corrected milk yields (Corrected for lactation number, farm, calving order) (DeGaris et al., 2008)



Disease and calcium metabolism



Increased exposure to transition also reduced Risk of Culling and Death (DeGaris et al., 2010 Aust Vet J)



Martinez et al., 2012

Table 2. Incidence and adjusted risk ratio (ARR) of metritis and puerperal metritis[1]

Item	Incidence, % (no./no.)	ARR (95% CI)	*P*-value
Metritis			
Ca status[2]			
Normocalcemia	15.8 (6/38)	Referent	
Subclinical hypocalcemia	63.9 (46/72)	3.24 (1.51–6.95)	<0.01
Parity			
Multiparous	40.5 (30/74)	Referent	
Primiparous	61.1 (22/36)	1.28 (0.96–1.69)	0.09
Risk group[3]			
Low risk	27.3 (15/55)	Referent	
High risk	67.3 (37/55)	1.80 (1.15–2.81)	<0.01
Puerperal metritis			
Ca status			
Normocalcemia	2.5 (1/38)	Referent	
Subclinical hypocalcemia	44.4 (32/72)	11.5 (1.57–83.6)	<0.02
Parity			
Multiparous	25.7 (19/74)	Referent	
Primiparous	38.9 (14/36)	1.32 (0.82–2.11)	0.24
Risk group			
Low risk	14.5 (8/55)	Referent	
High risk	45.4 (25/55)	1.79 (0.92–3.47)	0.08

[1]Metritis = cows with watery fetid vaginal discharge within the first 12 DIM; puerperal metritis = metritis concurrent with a rectal temperature ≥39.5°C.

[2]Normocalcemia = cows with serum Ca concentrations >8.59 mg/dL in the first 3 DIM; subclinical hypocalcemia = cows with at least 1 d with serum Ca concentration ≤8.59 mg/dL in the first 3 DIM.

[3]Low risk = normal calving; high risk = cows diagnosed with dystocia, twins, stillbirth, or retained fetal membranes.

Effects of prepartum dietary cation-anion difference intake on production and health of dairy cows: a meta-analysis

I.J. Lean,[1,2] J.E.P. Santos[3], E. Block[4], H.M. Golder,[1]

[1]*Scibus*, Camden, NSW, Australia,

[2]Dairy Science Group, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, NSW, Australia,

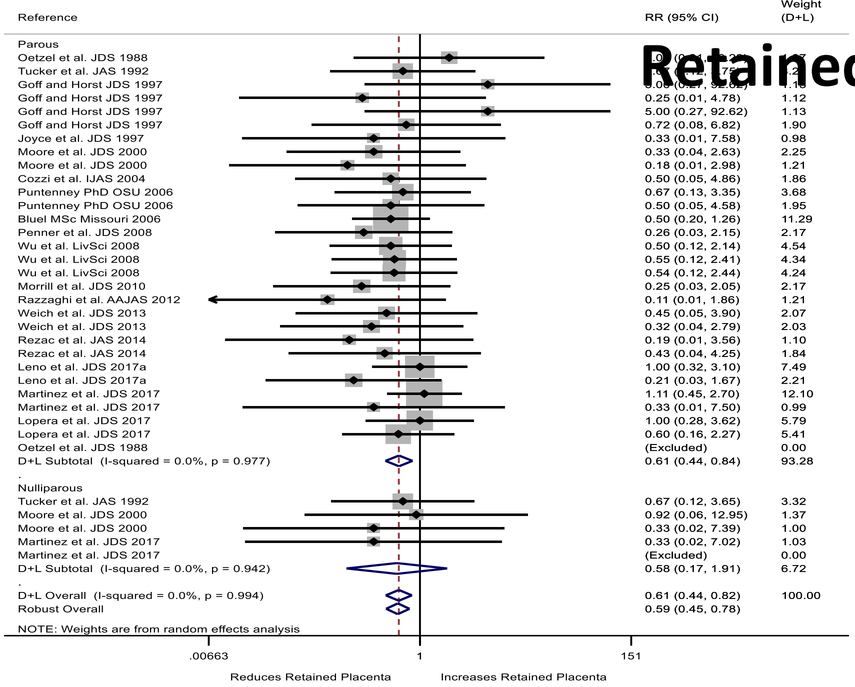
[3]Department of Animal Sciences, University of Florida, Gainesville, FL,

[4]Arm & Hammer Animal Nutrition, Princeton, NJ, 08543

Mineral composition of diets offered to prepartum cows and intake of minerals according to treatment group[1]

	Control				Treated		
Item	Mean	SD	Minimum and Maximum		Mean	SD	Minimum and Maximum
Content, % DM							
Ca	0.85	0.33	0.27 to 1.56		0.95	0.37	0.27 to 1.66
P	0.37	0.09	0.23 to 0.66		0.38	0.10	0.24 to 0.67
Mg	0.32	0.10	0.19 to 0.68		0.38	0.10	0.16 to 0.51
K	1.56	0.63	0.93 to 3.22		1.41	0.44	0.93 to 3.06
Na	0.22	0.28	0.03 to 1.60		0.16	0.08	0.03 to 0.44
S*	27.08	8.80	0.16 to 0.71		44.01	18.28	0.11 to 0.71
Cl*	53.40	24.01	0.20 to 1.11		93.57	36.63	0.11 to 3.19
DCAD[2], mEq/kg*	212.55	209.77	-62.38 to 1094.43		-50.78	121.70	-245.92 to 438.65
Intake, g/d							
Ca	98.71	45.52	23.30 to 223.08		107.46	52.82	20.87 to 272.24
P	41.80	10.72	23.66 to 66.46		41.20	12.50	21.47 to 81.78
Mg	36.95	13.95	18.20 to 82.32		36.87	13.20	17.48 to 73.44
K	172.97	56.54	64.29 to 372.69		154.77	51.05	55.37 to 369.65
Na	24.07	25.55	1.97 to137.60		18.13	10.47	1.70 to 44.28
S*	27.08	8.80	12.34 to 55.11		44.01	18.28	13.00 to 101.53
Cl*	53.40	24.01	7.87 to 136.94		93.57	36.63	29.36 to 177.12
DCAD[3], net Eq/d*	2.28	1.90	-0.69 to 9.41		-0.64	1.32	-2.80 to 3.95

Retained Placenta

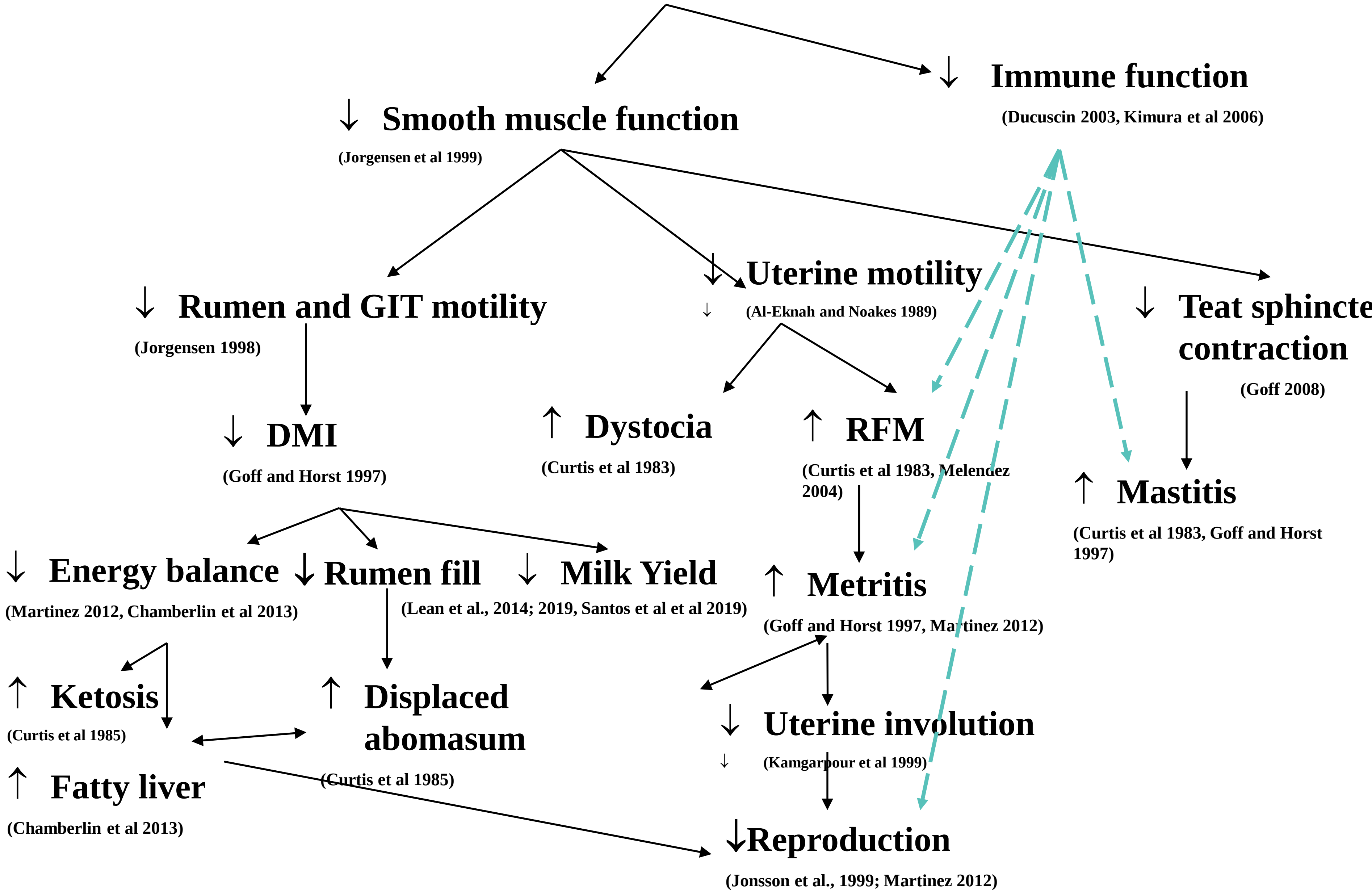


Other Outcomes: Robust regression

Variable	RR or OR (95% CI)	*P*-Value
Clinical Hypocalcemia	0.597 (0.440 to 0.810)	<0.01
Retained Placenta	0.590 (0.448 to 0.779)	0.02
Metritis	0.457* (0.277 to 0.756)	0.01
Mastitis	0.897* (0.536 to1.50)	0.63
Displaced Abomasum	0.903* (0.465 to 1.752)	0.73
Total Disease	0.609* (0.487 to 0.726)	<0.01

OR – *

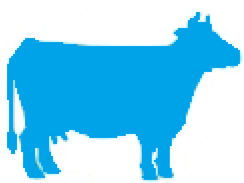
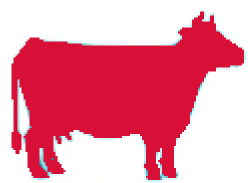
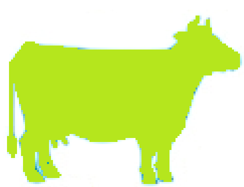
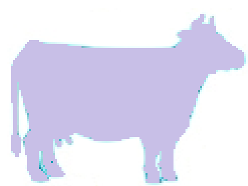
Hypocalcaemia



Skeleton and Vitamin D in dairy cattle

Transition Cattle: (Martinez et al 2018 ab: Rodney et al 2018 ab)

- Oral calcidiol and DCAD treatment for 21 days prepartum, common diet from calving to 49 DIM, diets were balanced for metabolizable protein
- 79 transition cows and heifers

	Negative DCAD – 130 meq/kg	Positive DCAD +130 meq/kg
Cholecalciferol Vitamin D_3 3 mg/d		
Calcidiol 25-OH-D_3 3 mg/d		

Detailed Physiology
Milk Data
Disease
Monitored daily to 30 DIM.
Reproduction

Results: Disease (Martinez et al., 2018)

- Incidence of **clinical hypocalcemia was 0% in negative DCAD and 23.1%** in **positive DCAD**.
- **Subclinical** hypocalcemia (iCa <1.06mM) was reduced with a negative DCAD diet
  - **Negative** DCAD at 0 and 1 DIM (**20%, 34.3%) v positive** DCAD (**69.3% and 76.5%)**
- Calcidiol reduced incidence of **retained placenta (2.5% v 30.8%) and metritis (23.1% vs 42.2%)**.
- **Cows fed negative DCAD and calcidiol** had reduced morbidity compared with all 3 other treatments.

DCAD and Vitamin D

Table 6. Cox's proportional hazard model for time to pregnancy in cows fed 2 levels of DCAD and 2 sources of vitamin D prepartum[1]

	Days to pregnancy[2]				
Variable	Median (95% CI)	Mean ± SEM	Pregnant, %	AHR[3] (95% CI)	*P*-value
DCAD[4]					
Positive	144 (79 to 179)	151 ± 13	76.5	Referent	—
Negative	150 (133 to 183)	165 ± 11	80.2	0.84 (0.50 to 1.39)	0.49
Vitamin D					
Cholecalciferol	163 (135 to 183)	166 ± 12	71.2	Referent	—
Calcidiol	144 (110 to 150)	150 ± 11	84.2	1.55 (0.92 to 2.61)	0.10
Parity					
Parous	163 (144 to 184)	172 ± 11	73.1	Referent	—
Nulliparous	114 (76 to 144)	130 ± 10	82.9	1.76 (1.03 to 3.02)	0.04

[1]Prepartum cows at 252 d of gestation were fed diets with either positive (+130 mEq/kg) or negative (−130 mEq/kg) DCAD and containing either 3 mg of cholecalciferol or 3 mg of calcidiol.

[2]Pregnancy was based on the diagnosis on d 70 after each AI within the first 305 DIM.

[3]AHR = adjusted hazard ratio.

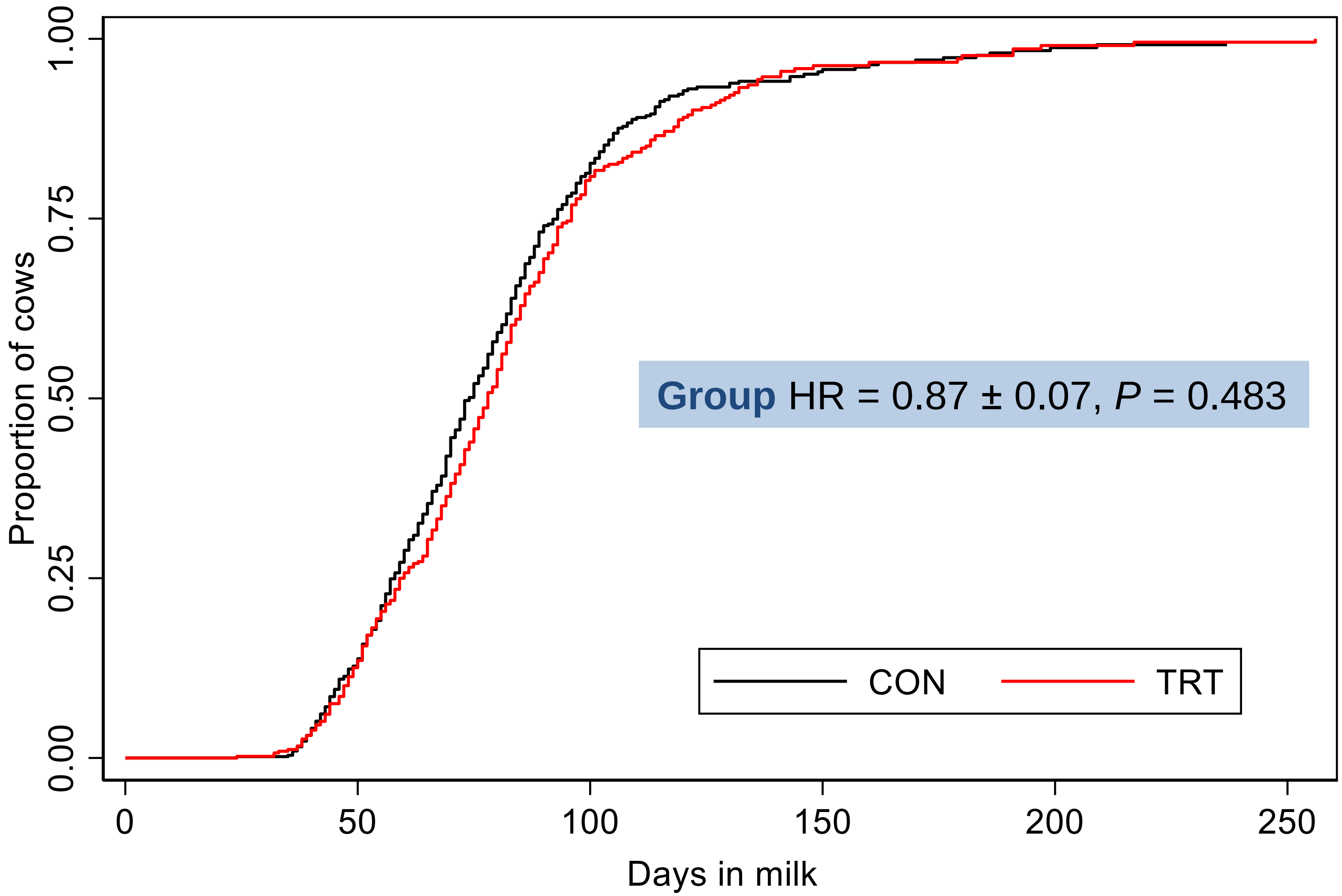
[4]Interaction between level of DCAD and source of vitamin D was not significant and dropped from final model.

Calcidiol and reproduction Golder et al., (in review)

- Cows from **4 dairies** with prepartum transition diets formulated to have negative DCAD
- **Experiment 1;** cows were assigned to **Control [n = 645; no 25-(OH)D3] or Treatment [TRT; n = 537; 2 mg/d of 25-(OH)D3 from ~21 d prepartum to parturition and 1 mg/d in lactation.**
- **Experiment 2**; cows (n = 2,064; median 147 DIM) in 4 groups and monitored Exp 1 to the end of that lactation (L1), subsequent transition (~21 d prepartum to parturition) and lactation (L2).
- Groups were as follows, with the mg/d of 25-(OH)D3 fed indicated in brackets for L1, *transition*, and L2, respectively: (A) Control-control (**CON-CON; 0 – 0 – 0)**, (B) Treatment-treatment (**TRT-TRT; 1 – 2 – 1**), (C) Control-treatment (**CON-TRT; 0 – 2 – 1**), and (D) Treatment-control (**TRT-CON; 1 – 0 – 0**).

Exp 1: Days to first breeding – Golder et al. (In Review)

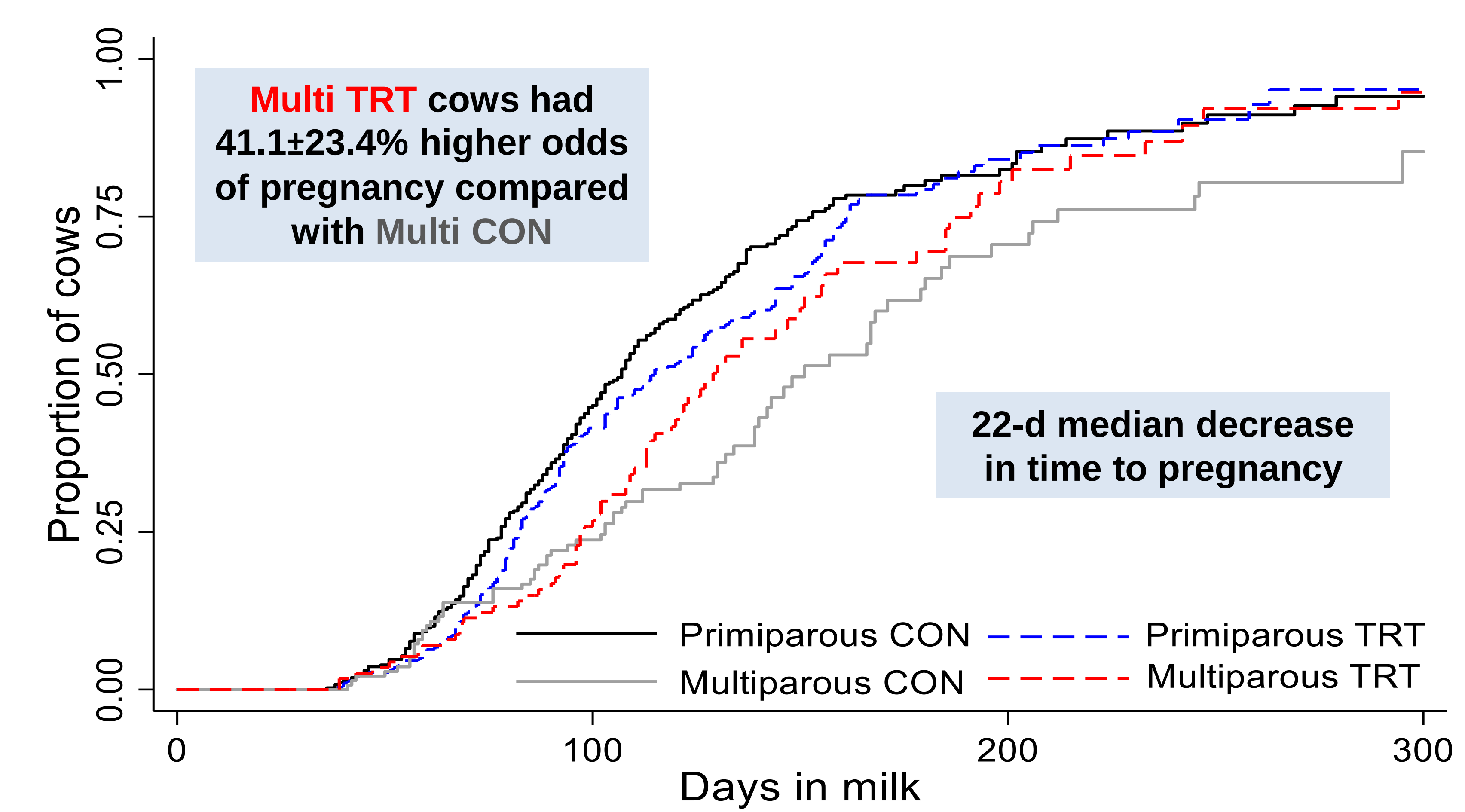
Days spent on transition HR = 1.01 ± 0.003, 95% CI = 1.00 to 1.01; *P* = 0.010



For example, cows that had 20 days on transition, had approximately a 15% increase in probability of being bred per d.vs those with zero days

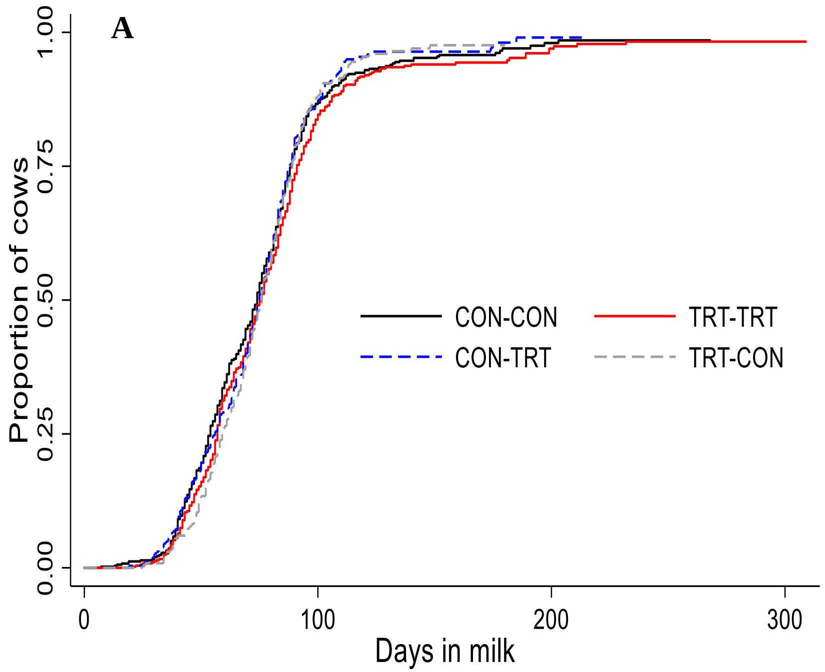
Exp 1: Time to Pregnancy – Golder et al. (Under Review)

Days spent on transition HR = 1.00 ± 0.004, 95% CI = 0.99 to 1.01; *P* = 0.587

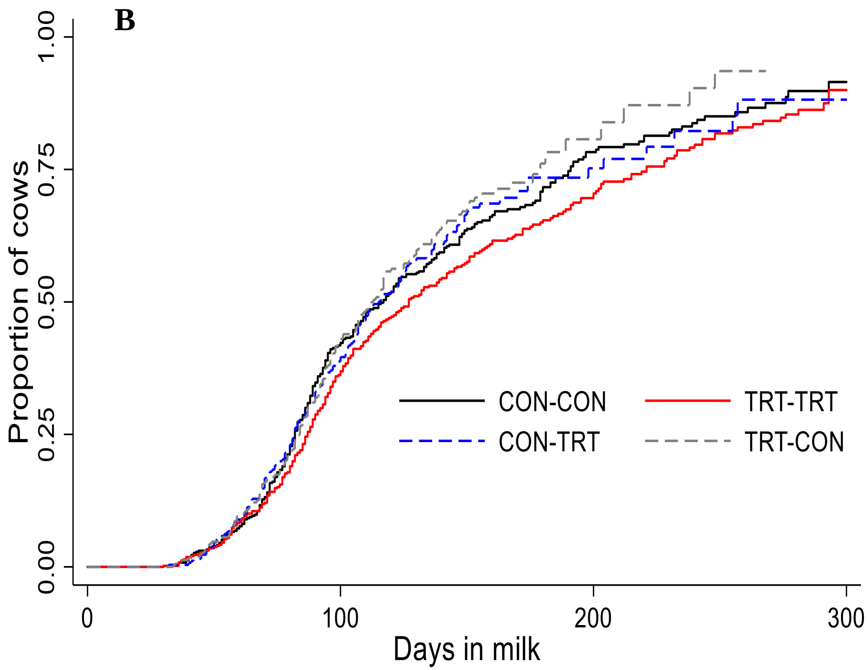


Exp 2: Golder et al. (Under Review)

Days to first breeding



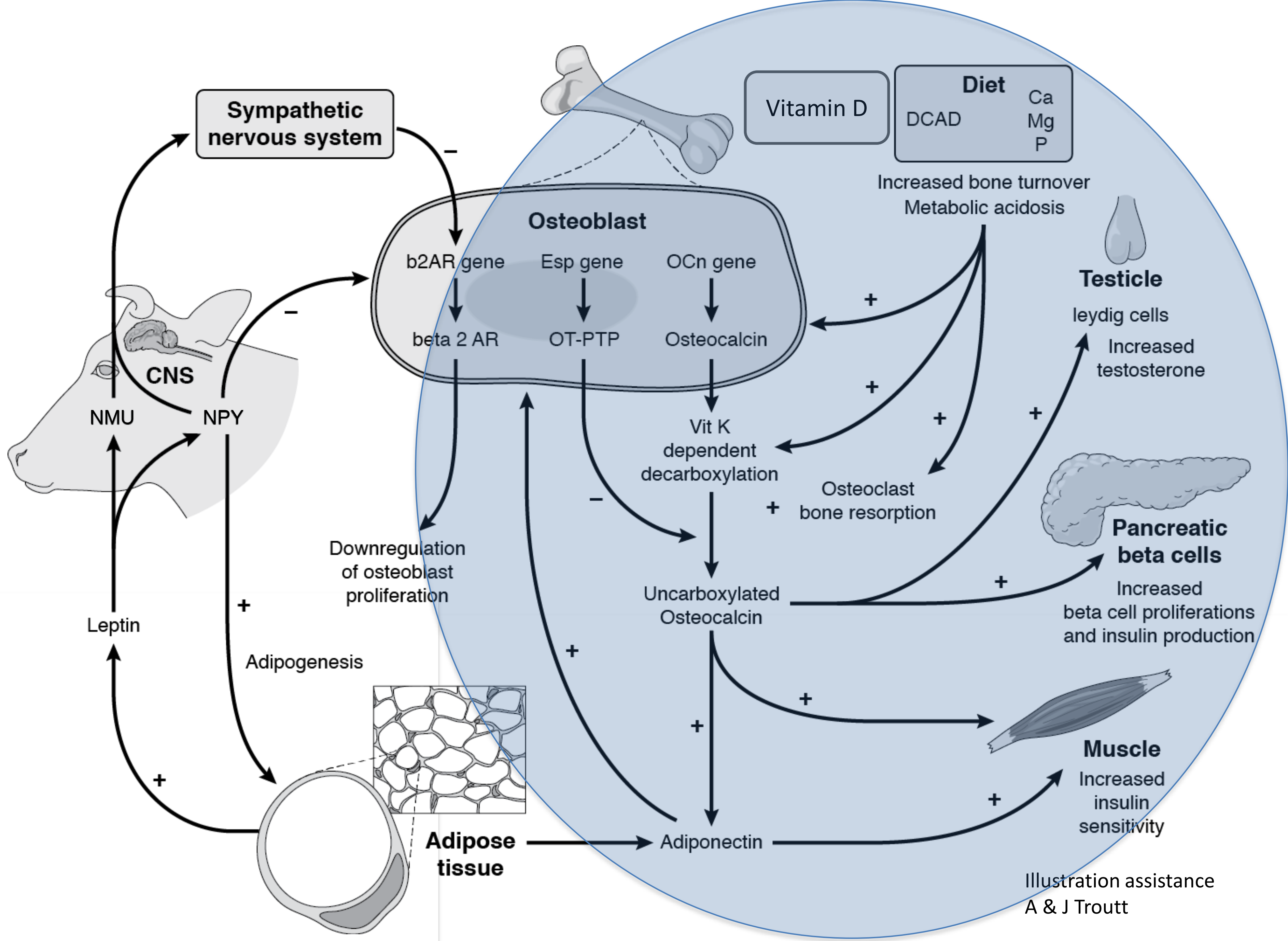
Days to pregnancy



- Group (*P* = 0.016)
- **TRT-TRT cows had between 15.5 and 28.9% lesser odds to be bred per d than all other groups**
- **Days on transition HR = 1.02 ± 0.003; *P* <0.001**

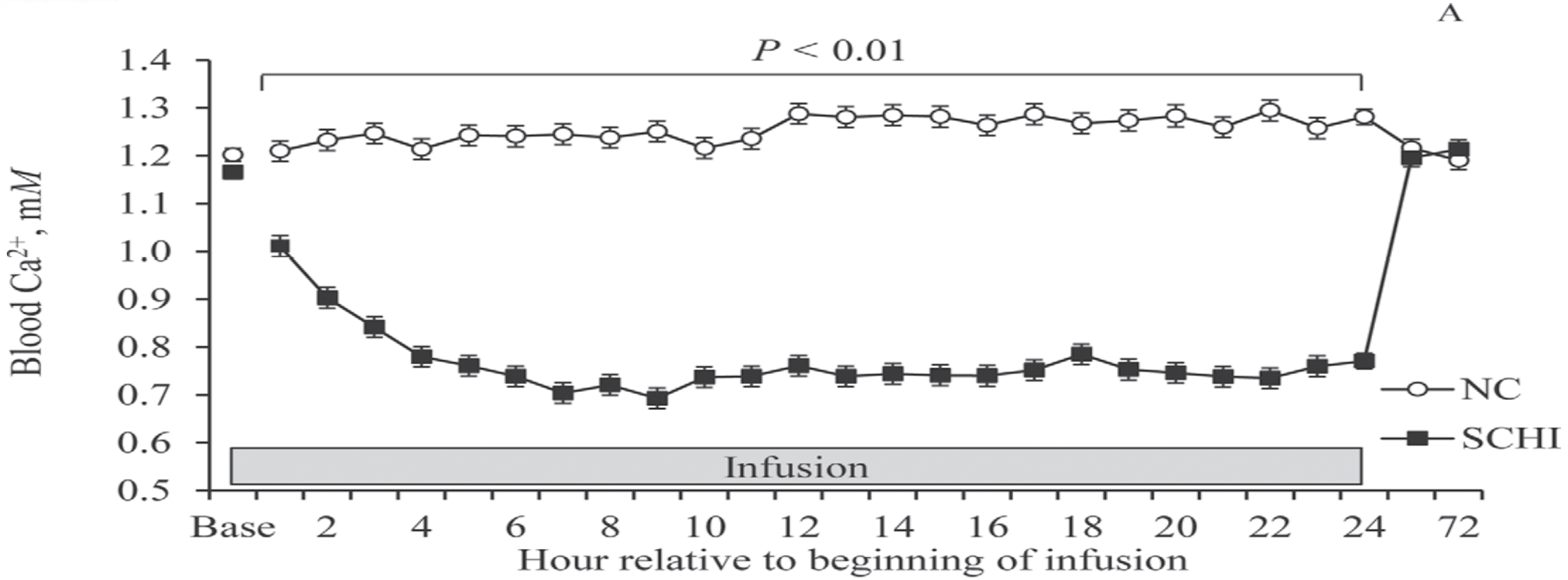
- Group (*P* = 0.067)
- CON-CON cows had 17±7.0% and TRT-CON cows 26.6±13.4% greater odds to be pregnant per d than **TRT-TRT** cows
- **Days on transition HR = 1.007 ± 0.004; *P* = 0.064**

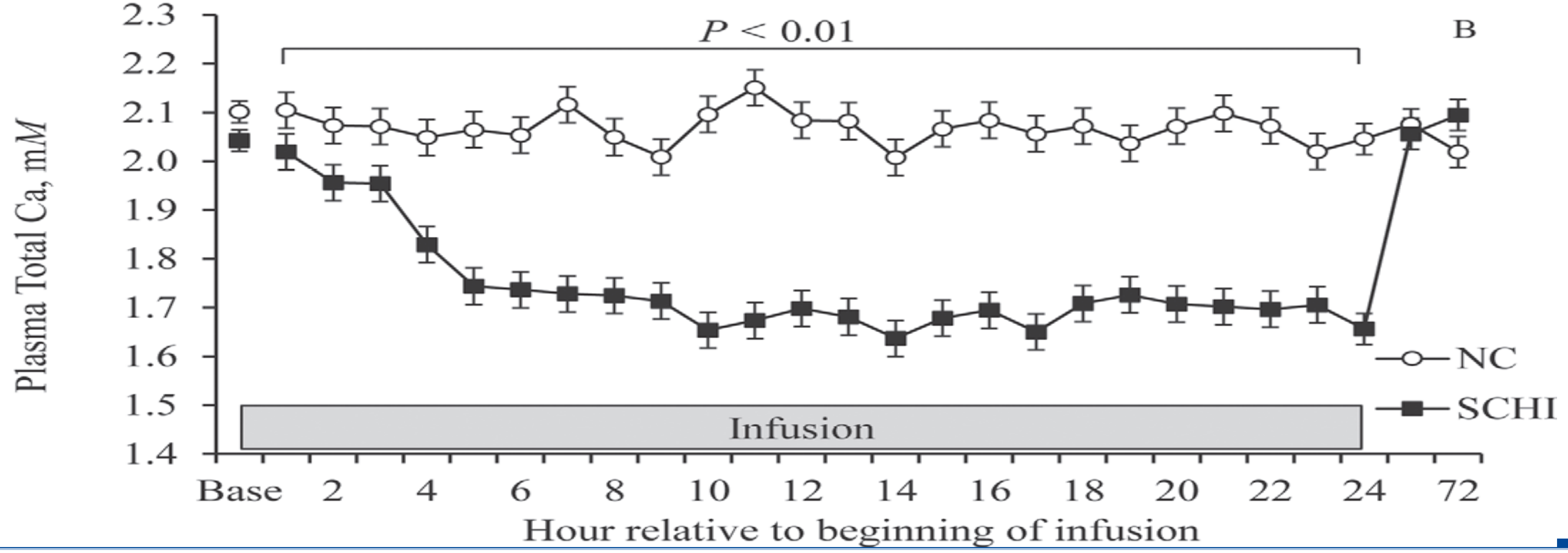
FURTHER MECHANISM: SKELETON



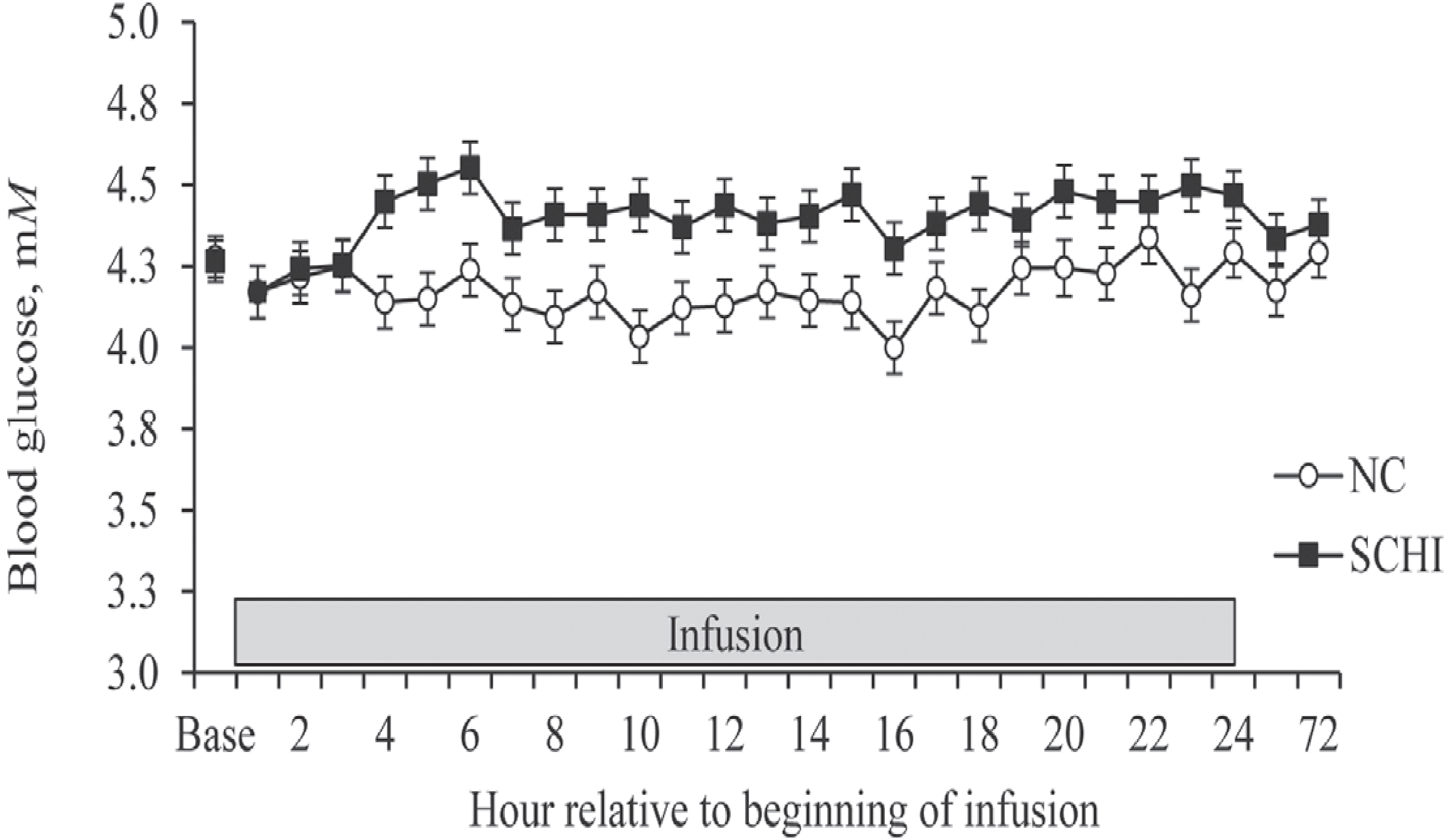
Sympathetic nervous system
Vitamin D
Diet
DCAD
Ca
Mg
P
Increased bone turnover
Metabolic acidosis
Osteoblast
b2AR gene
Esp gene
OCn gene
beta 2 AR
OT-PTP
Osteocalcin
Testicle
leydig cells
Increased testosterone
CNS
NMU
NPY
Vit K dependent decarboxylation
Osteoclast bone resorption
Downregulation of osteoblast proliferation
Pancreatic beta cells
Uncarboxylated Osteocalcin
Increased beta cell proliferations and insulin production
Leptin
Adipogenesis
Muscle
Increased insulin sensitivity
Adipose tissue
Adiponectin
Illustration assistance
A & J Troutt

Induced hypocalcaemia (Martinez et al., 2014)

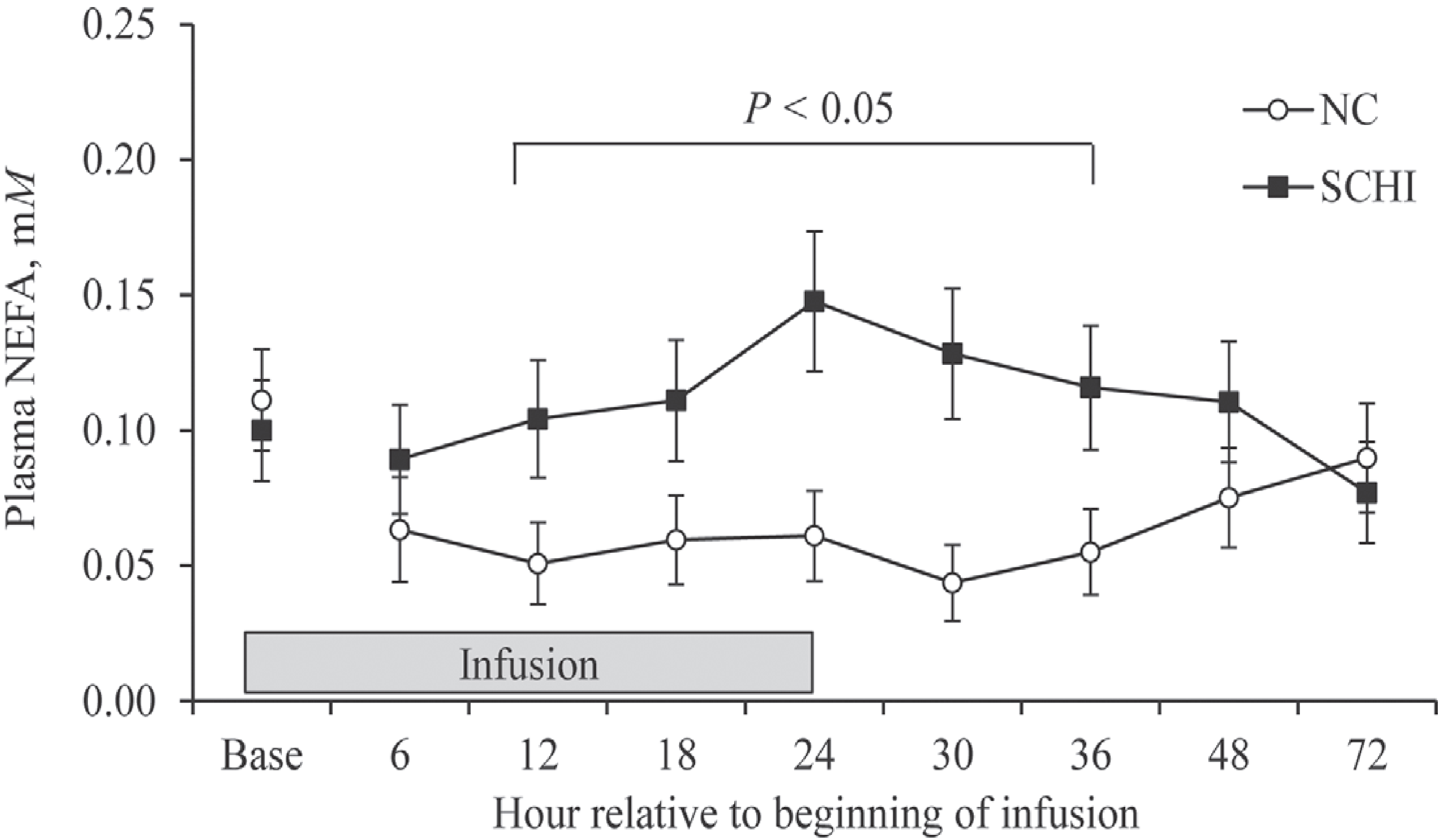




Induced hypocalcaemia (Martinez et al., 2014)



Induced hypocalcaemia (Martinez et al., 2014)



Time series cross-correlations between calcium and other metabolites measured at lags of 3.5 days (Lean et al., 2014)

Input variable	Output Variable	Lag*	Pooled effect size	P value	Heterogeneity I^2
Calcium	NEFA	-1	0.172	0.063	57%
Calcium	NEFA	0	-0.194	0.064	70%
Calcium	NEFA	5	-0.154	0.060	0%
Calcium	NEFA	6	0.182	0.048	0%
Calcium	BHB	-2	-0.140	0.028	0%
Calcium	Glucose	-2	0.146	0.030	0%
Calcium	Cholesterol	-2	0.149	0.023	4.6%
Calcium	Cholesterol	-1	-0.301	<0.001	0%
Calcium	Cholesterol	0	0.335	<0.001	56.8%
Osteocalcin*	**IGF1**	**0**	**>0.8**	**<0.001**	

***From Rodney et al 2018**

Conclusions

- Calcium and vitamin D metabolism are important modifiers of health and fertility
- Interventions manipulating these ie negative DCAD diets and calcidiol can help
- Parity effects may be important in modifying intervention responses
- More work is required on these to optimize responses and consider parity implications

Acknowledgements

- *Scibus*
- The University of Sydney
- Dairy Australia
- DSM Nutritional Products
- Arm and Hammer Animal Nutrition
- The University of Florida