Improving reproductive outcomes in ruminants through targeted nutritional intervention around conception

Professor Michael Friend
Director, Graham Centre
Where is the Graham Centre?
Lower Murray Darling Basin - value $10b pa - food & fibre

Grower Partnerships
- Growers
- Advisors
- Farming Systems Groups

Community and Environment Partnerships
- Local Land Services
- Landcare

Research Pathways
- Crops and pastures
- Crop protection and integrated pest management
- Fodder utilisation and animal production
- Animal health and welfare

Research Collaboration Partnerships
- CSIRO
- Universities
- International links

Funding Partnerships
- Research Development Corporations
- Australian Research Council

Private Sector Partnerships
- Agribusiness
- Consultants
- Commercial advisors
Key Objectives

• Increasing productivity gains and improving environmental sustainability in agriculture

• Improving food quality (nutritional & health attributes)
Research Collaboration

CSU (70 members)
  School of Agricultural & Wine Sciences
  School of Animal & Veterinary Sciences
  School of Biomedical Sciences

NSW DPI (25 members)
Post-graduate Students (80 students)
Research focus

• Livestock nutrition and reproduction (ruminant focus)
• Animal health & welfare
• Crop protection and integrated weed management
• Agronomy, soil science and hydrology
• Food science
Recent Investments in CSU Infrastructure

The National Life Sciences Hub - $45M

- State of the art molecular laboratories
- PC2 & 3 facilities, quarantine area (national critical infrastructure)
- Veterinary Diagnostic laboratory
- Contained glasshouses
- Rhizolysimeter
- Phytotron
- Commercial analytical laboratory
Nutritional intervention around conception to improve reproductive performance

- Reproductive performance a key determinant of productivity of many livestock enterprises
- ‘Reproductive wastage’ a major issue in extensive livestock systems
- Nutrition can have a major effect on reproductive performance
- Nutritional management around conception has received less attention than nutrition around parturition
  1. Nutritional management to improve ovulation rate and reduce embryo mortality
  2. Altering dietary omega fatty acid content to skew progeny sex ratio
Increasing reproductive performance by flushing and reducing embryo mortality
Background

• \( \uparrow \) ovulation rate = more twins = potential $

• Higher female body condition/liveweight; or

• Short-term ‘flushing’ (days 10-14 of the oestrous cycle)

• Limitations of flushing
  • Synchronization
  • Variable response
Liveweight and ovulation rate

Source: Lindsay (1988)
Lupin flushing

Percentage increase in twin ovulations of supplemented ewes over control ewes

Source: Lindsay (1988)
## Variable responses

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sheep breed (number of ewes per treatment)</th>
<th>Treatment (day of oestrous cycle)</th>
<th>Ovulation rate (mean ± s.e.*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control Supplemented/Treatment</td>
</tr>
<tr>
<td><strong>Lupin grain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Stewart, 1990) Experiment 1</td>
<td>Merino (n = 79)</td>
<td>1) 750g/hd oat grain day 9-12</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) 750g/hd lupins day 13-16</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) 750g/hd lupins day 9-12</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4) 750g/hd lupins day 9-16</td>
<td>1.14</td>
</tr>
<tr>
<td>(Stewart, 1990) Experiment 2</td>
<td>Merino (n = 150)</td>
<td>1) 750g/hd oat grain day 9-12</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) 750g/hd lupins day 9-12</td>
<td>1.29</td>
</tr>
<tr>
<td>(Stewart, 1990) Experiment 3</td>
<td>Merino (n = 70)</td>
<td>1) 750g/hd oat grain day 13-16</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) 750g/hd lupins day 13-16</td>
<td>1.39</td>
</tr>
<tr>
<td>(Stewart, 1990) Experiment 4</td>
<td>Merino (n = 70)</td>
<td>1) 750g/hd oat grain day 13-16</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) 750g/hd lupins day 13-16</td>
<td>1.35</td>
</tr>
<tr>
<td>(Gherardi and Lindsay, 1982)</td>
<td>Merino (n = 100)</td>
<td>Grazing natural pasture</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1) 750g/hd lupins day 6-14 (Oct)</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) 750g/hd lupins day 6-14 (Dec)</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) 750g/hd lupins day 6-14 (Jan)</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4) 750g/hd lupins day 6-14 (Mar)</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Source: Gulliver (2013). PhD thesis
Would flushing with green pasture be effective?

Oestrous-synchronised ewes grazed either
1. Dead pasture
2. Dead pasture + lupins (500g/d)
3. Green Lucerne, or
4. Green Chicory for 9 days prior to ovulation

Mean ovulation rate 2006-2008

Flushing effective with small amounts of live pasture

But still requires synchronisation!

How critical is timing of flushing for increasing ovulation rate?

Stewart et al (1990) – lupin flushing d13-16 reduced ovulation rate

Mean ovulation rate

<table>
<thead>
<tr>
<th></th>
<th>ME (MJ/kg)</th>
<th>CP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.6</td>
<td>7.1</td>
</tr>
<tr>
<td>Lupins</td>
<td>13.5</td>
<td>30.9</td>
</tr>
</tbody>
</table>

Plasma insulin increased by lupin feeding – increased glucose uptake by follicles?

Gulliver C (2013). PhD thesis
Can flushing naturally cycling ewes with lucerne ↑ lambs born?

- Ewes grazed lucerne or dead pasture for 7 days before and 7 days following males being introduced

Why not graze ewes on Lucerne beyond day 7?

In synchronised, pen-fed ewes (barley/lucerne pellet):

• Twice energy requirement = embryo mortality
• Sensitive to low progesterone Day 11-12

<table>
<thead>
<tr>
<th>Energy level (xM)</th>
<th>% ewes pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>200%</td>
<td>48 a</td>
</tr>
<tr>
<td>100%</td>
<td>68 b</td>
</tr>
<tr>
<td>25%</td>
<td>67 b</td>
</tr>
</tbody>
</table>

Source: Parr et al. 1987, 1992

Source: Parr et al. 1987, 1992
Embryo mortality and protein levels??

- Excess nitrogen/protein may cause embryo mortality
- More likely if extreme or sub-maintenance energy
- Is high protein in lucerne a concern?
Does the timing or quantity of lucerne fed influence embryo mortality and fetuses scanned?

Where insemination = Day 0:

1. 0-17 days Control pellets M
2. 0-17 days Lucerne M
3. 0-17 days Lucerne ad lib
4. 0-7 days Lucerne ad lib
5. -7-17 Lucerne ad lib

Synchronised ewes fed once daily
Feeding high levels of lucerne once daily to synchronised AI ewes reduces twinning rate

<table>
<thead>
<tr>
<th>Group</th>
<th>Proportion pregnant ewes with multiples</th>
<th>No. fetus per pregnant ewe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.34 b</td>
<td></td>
</tr>
<tr>
<td>0-17 M</td>
<td>0.32 b</td>
<td></td>
</tr>
<tr>
<td>0-17 ad lib</td>
<td>0.18 a</td>
<td></td>
</tr>
<tr>
<td>0-7 ad lib</td>
<td>0.22 ab</td>
<td></td>
</tr>
<tr>
<td>Minus 7-17 ad lib</td>
<td>0.23 ab</td>
<td></td>
</tr>
</tbody>
</table>

*Overall effect*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. fetus per pregnant ewe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance</td>
<td>1.33 b</td>
</tr>
<tr>
<td>Ad lib</td>
<td>1.21 a</td>
</tr>
</tbody>
</table>

What naturally cycling ewes grazing lush Lucerne?

Does grazing high levels of lucerne affect reproductive rate in naturally cycling ewes?

1. Dead pasture
2. Lucerne 7 days before to 7 days after ram introduction
3. Lucerne 7 days before and for 5 weeks after ram introduction
# Grazing trial results

<table>
<thead>
<tr>
<th></th>
<th>Dead pasture</th>
<th>Lucerne throughout</th>
<th>Lucerne to Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Returning to service (%)</td>
<td>19</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Non-pregnant (%)</td>
<td>12</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>No. fetuses/ewe joined</td>
<td>1.31 a</td>
<td>1.61 b</td>
<td>1.60 b</td>
</tr>
<tr>
<td>Lambs marked/ewe joined</td>
<td>0.96 a</td>
<td>1.14 b</td>
<td>1.15 b</td>
</tr>
</tbody>
</table>

Research gaps for flushing

• Are current feeding recommendations causing embryo losses (hidden wastage)?
• Are high levels of nutrition soon after fertilization only a problem for synchronized ewes?
• What are the mechanisms behind the flushing effect?
• What are the mechanisms behind high levels of nutrition and embryo mortality?
Omega fatty acids and reproduction

Eicosapentaenoic acid (EPA) – C20:5n-3
Omega-3 and Omega-6 in Plants

α-linolenic acid (ALA) - C18:3n-3 - Omega-3

Linoleic acid (LA) - C18:2n-6 - Omega-6
Sources of Omega-3 and Omega-6

**Omega-3**
- Pasture, vegetative cereals (including silage), forage legumes, linseed (flaxseed)
- α-linolenic acid (ALA) (C18:3n-3)
- Eicosapentaenoic acid (EPA) (C20:5n-3)

**Omega-6**
- Grains, soybean oil/meal, sunflower/safflower oil, cottonseed meal
- Linoleic acid (LA) (C18:2n-6)
- Arachidonic acid (AA) (C20:4n-6)
## Omega-3 in Animal Feed

<table>
<thead>
<tr>
<th>Forage</th>
<th>Type</th>
<th>Omega-3 (%)</th>
<th>Omega-6 (%)</th>
<th>n-6:n-3 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasture</td>
<td>Improved</td>
<td>47.9</td>
<td>10.0</td>
<td>0.21</td>
</tr>
<tr>
<td>Pasture</td>
<td>Lucerne</td>
<td>46.6</td>
<td>14.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Cereal</td>
<td>Oat/Pea</td>
<td>44.9</td>
<td>14.8</td>
<td>0.33</td>
</tr>
<tr>
<td>Pasture</td>
<td>Native/Improved</td>
<td>28.8</td>
<td>18.0</td>
<td>0.62</td>
</tr>
<tr>
<td>Silage</td>
<td>Ryegrass</td>
<td>49.1</td>
<td>3.59</td>
<td>0.31</td>
</tr>
<tr>
<td>Silage</td>
<td>Oats</td>
<td>37.1</td>
<td>13.3</td>
<td>0.36</td>
</tr>
<tr>
<td>Silage</td>
<td>Barley</td>
<td>31.4</td>
<td>12.8</td>
<td>0.41</td>
</tr>
<tr>
<td>Grain</td>
<td>Oats</td>
<td>1.1</td>
<td>33.7</td>
<td>31.5</td>
</tr>
<tr>
<td>Grain</td>
<td>Barley</td>
<td>4.3</td>
<td>47.6</td>
<td>11.0</td>
</tr>
<tr>
<td>Grain</td>
<td>Maize</td>
<td>11.0</td>
<td>52.5</td>
<td>4.8</td>
</tr>
<tr>
<td>Cottonseed</td>
<td>CSM</td>
<td>0.3</td>
<td>42.7</td>
<td>164.3</td>
</tr>
</tbody>
</table>
Metabolism to Prostaglandin

EPA - Omega-3

\[ \text{Removal of 2 double bonds} \]

\[ \text{PGF}_{3\alpha} \]

\[ \text{Inflammation} \]

AA - Omega-6

\[ \text{PGF}_{2\alpha} \]

\[ \text{Inflammation} \]
Research questions

– Potential inflammation with omega 6 (prostaglandin)
– Potential effects of omega FA on sex ratio
– Potential intergenerational effects
Omega-3 and Sex Ratio Studies

- 5 studies conducted between 2010-2013
- A total of 1524 ewes and 1736 lambs over 3 years
- Stemmed from initial PhD studies
Treatment Diets

**Omega-3**
90% Silage - 10% Molasses

**Omega-6**
70% Oats - 8% CSM

**Omega-6 : Omega-3**
0.93 : 1

**Omega-6 : Omega-3**
13.0 : 1
Blood Omega-3

Plasma Omega-3 ALA (% Total Fatty Acid)

Day of Feeding Experimental Diets

Joining

Silage (Omega-3)

Oats/CSM (Omega-6)
Prostaglandin Response

Time to Oestrus
Sex Ratio of Lambs – ewes fed pre and post conception

Is the effect pre conception only?

![Bar chart showing proportions of female lambs and p-values for different groups and conditions.](chart.png)
Outcomes

• Ewes fed Oats/CSM (high in omega-6) had:
  – more omega-6/less omega-3 in plasma
  – increased PG response to oxytocin
  – shorter time to oestrus
  – approximately 10-15% more female lambs
Can we alter Omega-3 in meat?

• Potential health benefits for humans consuming this meat
Changing Omega-3 in Lamb

Intergenerational Effects?

• Lamb fatty acids at birth
• Omega-3 metabolism
• Reproduction rate
Lamb Fatty Acids


![Bar chart showing the comparison of RBC EPA proportions between Dam fed Omega-3 and Dam fed Omega-6.]
Omega-3 Metabolism - Meat

![Graph showing EPA + DHA (mg/Serve of Lamb) for Dam fed Omega-3 and Dam fed Omega-6 with a p-value of 0.005]
Reproductive Rate


![Graph showing mean number of fetuses per ewe with dietary treatments and pregnancy status.

- **Ewes Pregnant**
  - Low n-6 (Silage): 1.44
  - High n-6 (Oats/CSM): 1.7

- **Ewes Joined**
  - Low n-6 (Silage): 1.3
  - High n-6 (Oats/CSM): 1.6

Significance levels:
- p = 0.002
- p = 0.007

Dietary Treatment fed to Dams
And possibly effects on subsequent generation on the effects of omega fatty acids on the sex ratio of their offspring

Research gaps

• Understanding the mechanism
• Omega FA effects on lamb survival
• Effect in unsynchronised ewes
• Target feeding
  – Type of grain and amount to feed
  – Length of time of feeding
  – Effect of pasture with different omega-3 content
• Intergenerational effects
  – Lifetime productivity
  – Interaction with lifetime diet
Other areas of research in animal science
Understanding toxic outbreaks: pastures and weeds

- Identification of species / causal compounds
- Validation of therapeutics

Veterinary diagnostic lab

- parasitology, pathology, micro, virology, molecular

Biosecurity research

- practices, attitudes and behaviours, emergency disease management
Cattle welfare

‘Calf alert’

Non-surgical de-sexing

telemetric monitoring of parturition

Kisspeptin/GnRH vaccine to prevent reproduction in males and females
Food science

• Meat science
• Improving grain quality (for humans and livestock)
• Improving byproduct quality (eg canola meal)
• Improving infant formula
Summary

- **Significant opportunities for research in production animal science**
- **Focus** on industry needs
- Build **capacity** through PhD and Postdocs
- Build multi-disciplinary **teams**
- **Excellence** in research leading to industry impact
An alliance between Charles Sturt University and NSW Department of Primary Industries

www.grahamcentre.net