Calm Merino ewes have a higher ovulation rate and more multiple pregnancies than nervous ewes

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In 1990, two selection lines of Merino sheep were established for low and high behavioural reactivity (calm and nervous temperament) at the University of Western Australia. Breeding records consistently showed that calm ewes weaned 10% to 19% more lambs than the nervous ewes. We hypothesise that calm ewes could have a higher ovulation rate than nervous ewes and/or calm ewes could have a lower rate of embryo mortality than nervous ewes. We tested these hypotheses by comparing the ovulation rate and the rate of embryo mortality between the calm and nervous lines before and after synchronisation and artificial insemination. Merino ewes from the temperament selection lines (calm, n = 100; nervous, n = 100) were synchronised (early breeding season) for artificial insemination (day 0) (intravaginal sponges containing flugestone acetate and eCG immediately after sponge withdrawal). On day-17 and 11 ovarian cyclicity and corpora lutea, and on days 30 and 74 pregnancies and embryos/foetuses were determined by ultrasound. Progesterone, insulin and leptin concentrations were determined in blood plasma samples from days 5, 12 and 17. Ovarian cyclicity before and after oestrus synchronisation did not differ between the lines, but ovulation rate did (day-17: calm 1.63; nervous 1.26; P < 0.01; day 11: calm 1.83; nervous 1.57; P < 0.05). Ovulation rate on day 11 in nervous ewes was higher than on day-17. Loss of embryos by day 30 was high (calm: 71/150; nervous: 68/130); but nervous ewes had a lower proportion (15/47) of multiple pregnancies compared with calm ewes (30/46; P < 0.01). Reproductive loss between days 30 and 74 represented 7.3% of the overall loss. Temperament did not affect concentrations of progesterone, but nervous ewes had higher insulin (32.0 pmol/l ± 1.17 SEM; P = 0.013) and lower leptin (1.18 μg/l ± 0.04 SEM; P = 0.002) concentrations than calm ewes (insulin: 27.8 pmol/l ± 1.17 SEM; leptin: 1.35 μg/l ± 0.04 SEM). The differences in reproductive outcomes between the calm and nervous ewes were mainly due to a higher ovulation rate in calm ewes. We suggest that reproduction in nervous ewes is compromised by factors leading up to ovulation and conception, or the uterine environment during early pregnancy, that reflect differences in energy utilisation.

Keywords: embryo, progesterone, insulin, leptin, Ovis aries

Implications
Breeding records of Merino sheep selected for low and high behavioural reactivity (calm and nervous temperament) at the University of Western Australia consistently showed that calm ewes weaned 10% to 19% more lambs than the nervous ewes. We found that the differences in reproductive outcomes between the calm and nervous ewes were mainly due to a higher ovulation rate (OR) in calm ewes, possibly due to the effects of metabolic hormones. Understanding why the reproductive outcome of these ewes is different will help to breed sheep better suited for the production system, improving their welfare.

Introduction
Since domestication, while natural selection has continued, subjective artificial selection has resulted in the adaptation of farmed livestock to their artificial environment. Two noticeable impacts of domestication are (1) a lower behavioural reactivity to the presence of humans or to a novel situation (being an important criterion for selection of domesticated animals) and (2) an increase in reproductive success (Price, 2002). It is generally accepted that stress has a tendency to...
Temperament and ovulation rate in sheep

impaired reproduction (Dobson et al., 2012), but important individual variation in the response to stress exists (Cockrem, 2013), possibly linked to coping styles or behavioural syndromes (Koolhaas et al., 2010). The link between low behavioural reactivity and high reproductive success is poorly understood. In this study, we explore the impact of differential selection for behavioural reactivity on reproductive success in sheep.

In 1990, two different selection lines of Merino sheep were established for low and high behavioural reactivity (also called calm and nervous temperament, respectively) at the University of Western Australia (Murphy et al., 1994; Blache and Bickell, 2010). In the early years of the selection, low behavioural reactivity was more strongly correlated with improved maternal behaviour and greater lamb survival than in the high behavioural reactivity ewes (Murphy et al., 1994; Murphy, 1999). After 10 to 13 years of selection, the differences in maternal behaviour and lamb survival between the two lines had dissipated (Bickell et al., 2010b), but breeding records of the two lines obtained over 4 years showed that 10% to 19% more lambs (to mated ewes) were weaned from the calm ewes than from the nervous ewes (Blache and Bickell, 2010). The higher reproductive success of the ewes of the calm line compared with those of the nervous line could be explained by diverse impacts of behavioural reactivity on the reproductive biology of each line (Blache and Bickell, 2010).

Previous investigations have shown that (1) 32 h after the withdrawal of progesterone sponges, calm ewes are more receptive in seeking males and receptive to approaches by males than nervous ewes (Gelez et al., 2003), (2) colostrum produced by calm ewes has more lactose, less fat and is less viscous than that from nervous ewes (Hart et al., 2006), (3) calm dairy ewes produce more milk than nervous ewes (Murray et al., 2009) and (4) milk from calm ewes has more protein than that of nervous ewes (Sart et al., 2004). While the studies listed above could contribute to the differences in lamb production between the two lines, an analysis of 2 years of data from routine ultrasound scanning of the two selection lines during mid pregnancy (60 to 90 days post mating) suggested that selection for behavioural reactivity could influence fertility and prolificacy. In fact, the analysis showed that ewes in the calm line carried more foetuses (1.39, n = 472) compared with nervous ewes (1.29, n = 302). The calm ewes carried a higher proportion of twins, 35.4% compared with 14.6% for the nervous ewes (P < 0.001; Blache and Bickell, 2010).

The differences in reproductive outcome between the low and high reactivity ewes could be due to differences in ovulation, ovulation rate and/or the maintenance of early pregnancy between the lines. To date, the influence of behavioural reactivity on any of these outcomes has not been investigated. We hypothesise that two factors may be responsible for the difference in the outcome of the early stages of the reproductive cycle between the two temperament selection lines. First, low behavioural reactivity ewes (calm ewes) could have a higher ovulation rate than high behavioural reactivity ewes (nervous ewes); and second, calm ewes could have a lower rate of embryo mortality than nervous ewes. We further hypothesise that the differential reproductive outcome is due to a more efficient in energy utilisation in calm ewes than in nervous ewes (Hafez and Lindsay, 1965). We tested these hypotheses by comparing the ovulation rate and the rate of embryo mortality between the calm and nervous lines before and after synchronisation and artificial insemination. After oestrus synchronisation, plasma concentrations of progesterone, insulin and leptin were measured to investigate the potential pathway(s) by which behavioural reactivity could influence these two early steps of the reproductive cycle.

Material and methods

This experiment was approved by the Animal Ethics Committee of the University of Western Australia (approval number 07/100/625). The experiment was conducted at Allandale Farm, which is located ~60 km north-east of Perth, WA (latitude 31°46′ south, longitude 116°29′ east). Merino ewes were selected from the calm (n = 100) and nervous (n = 100) selection lines. Selection was based on age (2.5 to 4.5 years), BW (±SD) (calm 57.2 ± 0.71 kg; nervous 57.7 ± 0.73 kg) and body condition score (±SD) (calm 2.85 ± 0.03; nervous 2.77 ± 0.03; based on a subjective scale where 1 is lean through to 5, which is fat; Jefferies, 1961), which did not differ between the lines.

Early in the breeding season (mid-January), the oestrus cycles of the ewes were synchronised for laparoscopic timed artificial insemination using intravaginal sponges containing 40 mg of fluogestone acetate (Chrono-gest® 40; Intervet, Bendigo, Victoria, Australia) for 14 to 15 days. Immediately after sponge withdrawal the ewes were injected with 200 IU of equine chorionic gonadotropin (eCG) (Folligon®; Intervet). After ~50 h of sponge removal the ewes were inseminated by laparoscopic intrauterine insemination by a veterinarian specialised in artificial insemination (AI). The ewes were sedated with 0.3 ml of xylazine hydrochloride (Rompun®, 20 mg/ml; Bayer, Gordon, NSW, Australia) i.m. Lignocaine hydrochloride (0.5 to 1 ml of Lignocaine®, 20 mg/ml; Illicit Veterinary Products, Smithfield, NSW, Australia) was injected s.c. in four sites around the insertion point of the laparoscopic probe 10 to 15 min before the procedure. At the end of the procedure, antiseptic (Terramycin®; Pfizer Australia, West Ryde, NSW, Australia) was sprayed on the insertion point. When released after the AI procedure, the ewes walked on their own without assistance and were fully recovered from sedation before being moved to their home paddock. In order to reduce the effects of individual ram fertility on the results, for each selection line, semen was collected from three rams and a minimum of 200 million sperm/dose of fresh semen was used. The day of insemination was designated as day 0. As insemination was carried out over 2 days, all days are quoted in relation to day 0 and may therefore be ±1 day. In total, 18 ewes were not inseminated because of either technical problems during synchronisation (n = 14) or due to the presence of lesions on the uterus (n = 4).
A further 17 ewes (calm: n = 9; nervous: n = 8) did not respond to the synchronisation treatment and were not inseminated.

On day-17 and 11 the corpora lutea (CL) were counted using real time B-mode ultrasound with a 7.5 MHz linear-array transrectal transducer (Aloka SSD 900; Aloka Co., Ltd, Tokyo, Japan), as described by Viñoles et al. (2010a). The number of embryos was recorded on day 30 using transrectal ultrasound and the number of foetuses on day 74 using transabdominal ultrasound with a 3.5 MHz convex transducer (Viñoles et al., 2010a). On days 5 (day of embryo arrival in the uterus), 12 (around maternal recognition of pregnancy) and 17 (folicular phase in non-pregnant ewes) blood samples were collected from the jugular vein to determine the concentrations of progesterone, insulin and leptin. Plasma was obtained by centrifugation of the samples immediately after collection and then stored at −20°C within 2 h. Ewes that had progesterone levels of >3.18 nmol/l on day 17 were considered to be pregnant and ewes with progesterone concentrations <3.18 nmol/l were considered to have returned to heat (Susmel and Piasentier, 1992). Ultrasound data from day 30 confirmed that ewes classified as having returned to heat were not pregnant.

**Hormone assays**

All hormone concentrations were measured in a single radioimmunoassay for each hormone using techniques validated for sheep samples. Plasma progesterone was assayed in duplicate using Active<sup>®</sup> Progesterone RIA DSL-3900 kits (Diagnostic Systems Laboratories Inc., Webster, TX, USA). Quality control samples containing endogenous progesterone (low 3.47 nmol/l and high 27.9 nmol/l) were assayed in duplicates at both the beginning and end of the assay. The intra-assay CV were 2.7% and 3.1%, respectively. The analytical detection limit of the assay was 1.27 nmol/l. Plasma insulin was assayed in duplicate by a double-antibody radioimmunoassay (Tindal et al., 1978). All samples were processed in a single assay and the limit of detection was 3.59 pmol/l. The assay included six replicates of three control samples, which were used to estimate the intra-assay CV of 6.6% at 16.1 pmol/l, 4.3% at 27.5 pmol/l and 4.9% at 48.9 pmol/l. Plasma leptin was measured in duplicate by a double-antibody radioimmunoassay developed for sheep in our laboratory and described in detail by Blache et al. (2000). All samples were processed in a single assay and the limit of detection was 0.10 μg/l. The assay included six replicates of three control samples, which were used to estimate the intra-assay CV of 6.3% at 0.54 μg/l, 4.8% at 0.97 μg/l and 3.8% at 1.85 μg/l.

**Statistical analysis**

Data were analysed using the SAS Systems programs (SAS 9.2V; SAS Institute Inc., Cary, NC, USA). Categorical data (ovulation rate, prolificacy and pregnancy rates) were analysed using $\chi^2$ procedure in SAS. Ovulation rate (OR; 1 or ≥2), pregnancy (0 or 1) and prolificacy (1 or ≥2) were analysed. The fixed effect in the model was temperament (calm or nervous). To analyse embryo losses, ewes were allocated into nine categories according to their reproductive outcome on the basis of possible combination of losses among the number of corpora lutea (CLs), number of embryos observed at day 30 and number of foetuses observed at day 74 of pregnancy, and the number of ewes in each category according to their temperament.
of significance was considered to be $P < 0.05$, and tendency as $0.05 < P < 0.10$.

Results

Ovarian cyclicity and ovulation rate
Before oestrus synchronisation (day-17) there was no difference between the selection lines in the proportion of ewes that were ovulating (64/182, 35.2%), but there was a difference in the ovulation rate between the selection lines. Calm ewes had a higher ovulation rate than nervous ewes ($P = 0.003$; Table 2). On day 11 the proportion of ewes that had ovulated in response to the synchronisation treatment was not different between the two lines (165/182, 90.7%). The average ovulation rate was higher in the synchronised cycle than in the previous cycle in both the calm (+12.3%) and nervous (+24.6%) lines, but the increase was significant only in nervous ewes ($P = 0.01$). On day 11 the ovulation rate was again higher in the calm ewes than the nervous ewes ($P = 0.03$; Table 2).

Pregnancy and prolificacy
On day 30 there was no difference in the proportion of ewes that were pregnant (calm 46/82, 56.1%; nervous 47/83, 56.6%) as detected by ultrasonography. There was a difference in the average litter size with calm ewes carrying more embryos than nervous ewes ($P = 0.001$; Table 3). On day 74 there was no difference in the proportion of ewes that were pregnant (calm 45/82, 54.9%; nervous 43/83, 51.8%). The average litter size of the calm line on day 74 was still higher, due to more multiple pregnancies, compared with the nervous line ($P = 0.03$; Table 3).

Loss of reproductive potential
Overall, there were fewer embryos present on day 30, and fewer foetuses on day 74, than expected from the number of CLs present on day 11 (Tables 2 and 3). On day 30, about half of the potential embryos had been lost, and although there was no difference in the proportion of overall lost embryos between the selection lines (calm 47.3%; nervous 52.3%), the ewes of the nervous line had proportionally fewer twin

Table 2 The number and percentage of calm and nervous ewes (Ovis aries) presenting 0, 1 or ≥2 corpora lutea (CL), number and percentage of ewes ovulating, total number of CL and ovulation rate on day-17 and 11 (day 0 = timed artificial insemination)

<table>
<thead>
<tr>
<th></th>
<th>Day-17</th>
<th></th>
<th>Day 11</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calm (n = 91)</td>
<td>Nervous (n = 91)</td>
<td>Calm (n = 82)</td>
<td>Nervous (n = 83)</td>
</tr>
<tr>
<td>Ewes with CL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>61</td>
<td>67.0</td>
<td>57</td>
<td>62.6</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>12.1</td>
<td>25</td>
<td>27.5</td>
</tr>
<tr>
<td>≥2</td>
<td>19</td>
<td>20.9</td>
<td>9</td>
<td>9.9</td>
</tr>
<tr>
<td>Ewes ovulating</td>
<td>30</td>
<td>33.0</td>
<td>34</td>
<td>37.4</td>
</tr>
<tr>
<td>Total no. of CL</td>
<td>49</td>
<td>43</td>
<td>150</td>
<td>130</td>
</tr>
<tr>
<td>Ovulation rate</td>
<td>1.63a</td>
<td>1.26b</td>
<td>1.83a</td>
<td>1.57b</td>
</tr>
</tbody>
</table>

A,BValues within day with different superscript letters differ significantly at $P < 0.01$ (day-17).
A,bValues within day with different superscript letters differ significantly at $P < 0.05$ (day 11).

Table 3 The number and percentage of calm and nervous ewes (Ovis aries) presenting 0, 1 or ≥2 embryos or foetuses, number and percentage of ewes pregnant, total number of embryos or foetuses and prolificacy on days 30 and 74 (day 0 = timed artificial insemination)

<table>
<thead>
<tr>
<th></th>
<th>Day 30</th>
<th></th>
<th>Day 74</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calm (n = 82)</td>
<td>Nervous (n = 83)</td>
<td>Calm (n = 82)</td>
<td>Nervous (n = 83)</td>
</tr>
<tr>
<td>Ewes with embryos or foetuses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>36</td>
<td>43.9</td>
<td>36</td>
<td>43.4</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>19.5</td>
<td>32</td>
<td>38.6</td>
</tr>
<tr>
<td>≥2</td>
<td>30</td>
<td>36.6</td>
<td>15</td>
<td>18.1</td>
</tr>
<tr>
<td>Ewes pregnant</td>
<td>46</td>
<td>56.1</td>
<td>47</td>
<td>56.6</td>
</tr>
<tr>
<td>Total no. of embryos or foetuses</td>
<td>79</td>
<td>62</td>
<td>72</td>
<td>58</td>
</tr>
<tr>
<td>Prolificacy</td>
<td>1.72a</td>
<td>1.32b</td>
<td>1.60a</td>
<td>1.35b</td>
</tr>
</tbody>
</table>

A,BValues within day with different superscript letters differ significantly at $P < 0.01$ (day 30).
A,bValues within day with different superscript letters differ significantly at $P < 0.05$ (day 74).
pregnancies than would be expected from the CLs present on day 11. Between days 30 and 74 fewer embryos were lost than between days 0 and 30 (calm 4.7%; nervous 3.1%) and single and multiple pregnancies were equally affected. Of the total reproductive wastage, 92.7% occurred between days 0 and 30 and 7.3% between days 30 and 74, but the overall wastage rate in each period was similar in the two selection lines.

**Progesterone**

The effect of temperament on plasma concentrations of progesterone was not significant (P = 0.79), but day, pregnancy status and the interaction day × pregnancy status were significant (P < 0.0001) (Table 4). The proportion of ewes with late reproductive loss (pregnant on day 17, with progesterone >3.18 nmol/l, and not pregnant on day 30) was higher in the calm ewes than the nervous ewes (P = 0.02) (Table 5).

**Insulin**

Temperament (P = 0.013) and day (P < 0.0001) affected the plasma concentrations of insulin but not pregnancy status, which was taken out of the model (Table 6). The interaction temperament × day was not significant (P = 0.26). The ewes of the calm line had lower plasma concentrations of insulin (27.8 pmol/l ± 1.17 SEM) than the ewes of the nervous line (32.0 pmol/l ± 1.17 SEM) regardless of their pregnancy status.

**Leptin**

Temperament (P = 0.002) and day (P < 0.0001) had a significant effect on the plasma concentration of leptin, but not pregnancy status, which was taken out of the model (Table 6). The interaction temperament × day was not significant (P = 0.25). The ewes of the calm line had higher plasma concentrations of leptin (1.35 μg/l ± 0.04 SEM) than the ewes of the nervous line (1.18 μg/l ± 0.04 SEM) regardless of their pregnancy status.

**Discussion**

The first part of the hypothesis that selection for behavioural reactivity in Merino ewes would affect ovulation rate was supported; the calm ewes had a higher ovulation rate and more multiple pregnancies than the nervous ewes. The second part of the hypothesis that the level of behavioural reactivity would affect conception or embryo loss was not supported, although the timing of reproductive loss seemed to be affected. The mechanism by which behavioural

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**Table 4** Mean concentrations of progesterone (nmol/l) for ewes (Ovis aries) on days 5, 12 and 17 after timed artificial insemination (TAI), according to pregnancy status on days 17 and 30 after TAI

<table>
<thead>
<tr>
<th>Pregnancy status1</th>
<th>(n = 87)</th>
<th>Late reproductive loss2</th>
<th>(n = 43)</th>
<th>Early reproductive loss3</th>
<th>(n = 29)</th>
<th>RMSE</th>
<th>P (pregnancy status)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 5</td>
<td>5.6A</td>
<td>4.0A</td>
<td>5.3A</td>
<td>0.73</td>
<td>0.0589</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 12</td>
<td>9.9B</td>
<td>8.9B</td>
<td>7.2B</td>
<td>0.73</td>
<td>0.0023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 17</td>
<td>10.9B</td>
<td>10.0B</td>
<td>8.0C</td>
<td>0.73</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RMSE = root mean square error.

1Pregnant: pregnant on days 17 and 30.
2Late reproductive loss: pregnant on day 17 and not pregnant on day 30.
3Early reproductive loss: not pregnant on days 17 and 30. Ewes that had progesterone levels of >3.18 nmol/l on day 17 were considered to be pregnant at that time.

a,bValues within column with different superscript letters differ significantly at P < 0.01.

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**Table 5** The number of calm and nervous ewes (Ovis aries) classified as pregnant (pregnant on both days 17 and 30), late reproductive loss (pregnant on day 17 and not pregnant on day 30) and early reproductive loss (not pregnant on days 17 and 30) (day 0 = timed artificial insemination)

<table>
<thead>
<tr>
<th>Pregnancy status1</th>
<th>Calm (n = 79)</th>
<th>Nervous (n = 80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n %</td>
<td>n %</td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>45 57.0</td>
<td>42 52.5</td>
</tr>
<tr>
<td>Late reproductive loss</td>
<td>26 a 32.9</td>
<td>17 b 21.3</td>
</tr>
<tr>
<td>Early reproductive loss</td>
<td>8 a 10.1</td>
<td>21 b 26.3</td>
</tr>
</tbody>
</table>

a,bValues within a row with different superscripts differ significantly at P < 0.05.

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**Table 6** Mean concentrations of insulin (pmol/l) and leptin (μg/l) for calm and nervous ewes (Ovis aries) on days 5, 12 and 17 after timed artificial insemination

<table>
<thead>
<tr>
<th></th>
<th>Calm</th>
<th>Nervous</th>
<th>RMSE</th>
<th>P (temperament)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>31.8 a</td>
<td>37.8 a</td>
<td>12.05</td>
<td>0.0093</td>
</tr>
<tr>
<td>Day 12</td>
<td>25.7 b</td>
<td>30.4 b</td>
<td>12.05</td>
<td>0.0410</td>
</tr>
<tr>
<td>Day 17</td>
<td>26.0 b</td>
<td>27.7 b</td>
<td>12.05</td>
<td>0.4631</td>
</tr>
<tr>
<td>Leptin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>1.40 A</td>
<td>1.26 A</td>
<td>0.253</td>
<td>0.0219</td>
</tr>
<tr>
<td>Day 12</td>
<td>1.41 A</td>
<td>1.26 A</td>
<td>0.253</td>
<td>0.0143</td>
</tr>
<tr>
<td>Day 17</td>
<td>1.24 B</td>
<td>1.02 B</td>
<td>0.253</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

RMSE = root mean square error.

a,bValues within column with different superscript letters differ significantly at P < 0.05.

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van Lier, Hart, Viñoles, Paganoni and Blache
reactivity affects ovulation rate is not understood but our
data lead us to suggest that rather than a direct effect of the
stress axis on the ovarian function it could be due to a more
efficient in energy utilisation in calm ewes than in nervous
ewes as hypothesised.

Ovulation can be directly blocked by stress because
increased cortisol secretion can reduce LH pulsatility and block
or delay the LH surge (Breen et al., 2005). When under stress,
the nervous ewes secrete more cortisol than the calm
ewes (Hawken et al., 2013), but the resting activity of the
hypothalamus-pituitary-adrenal axis is similar in ewes selected
to be calm or nervous (Rietema et al., 2015). Although we did
not measure cortisol, it is unlikely that the lower reproductive
performance of the nervous ewes was due to activation of the
stress axis, as there was no difference in the number of
ewes of each line ovulating, nor any difference in the fertility
to timed artificial insemination. Therefore, behavioural
reactivity seems not to affect ovulation per se.

The number of gonadotropin-dependent follicles becoming
ovulatory follicles depends on the duration of the window
of FSH above threshold values, the number of gonadotropin-dependent follicles present at the time of this
window or both (Scaramuzziet al., 2011). Reduced negative
feedback on the hypothalamus and pituitary due to lower
levels of oestrogens and/or inhibins can lead to a longer
duration of above threshold FSH levels. The higher leptin
concentrations in the calm ewes may have reduced aroma-
tase activity in the follicle, resulting in lower oestrogen pro-
duction (Kendall et al., 2004). In addition, leptin increases
the efficiency of the use of FSH by the follicles (Viñoles et al.,
2010b). While these mechanisms are theoretically sound,
cautions should be taken with this interpretation as no blood
samples were taken during the follicular phase before AI.

Ovulation rate after oestrus synchronisation increased in
nervous ewes and is influenced by eCG, although it must be
noted that ovulation rate before oestrus synchronisation was
higher in the calm ewes than in the nervous ones. Co-selection
for eCG sensitivity cannot be ruled out during temperament selection, nevertheless, the higher ovulation
rate in the nervous ewes was not accompanied with higher
proliferation. The increased in ovulation rate of the nervous
ewes in response to eCG may suggest that, in contrast to the
ewes of the calm line, ewes of the nervous line were not
ovulating at a rate close to their maximum genetic potential
when hormonal treatments were not applied.

As expected, we recorded the major part of reproductive
loss (>90%) before day 30 (Ashworth, 1995). However, the
timing differed between the two lines; more nervous ewes
either failed to conceive or lost more pregnancies before
day 17, whereas more calm ewes lost pregnancies after
day 17. It is unlikely that the nervous ewes were affected
by handling around AI because the proportion of ewes
ovulating did not differ between the lines and the ewes were
handled gently and only rough handling or the use of dogs
can affect maintenance of pregnancy during the first 20 days
of gestation (Doney et al., 1976). It is possible that the
higher plasma concentrations of leptin in calm ewes may
have led to better embryo survival (Sosa et al., 2006;
Viñoles et al., 2012), whereas the lower plasma concentrations
of leptin in nervous ewes may have affected the expression of
steroid receptors in the uterus, thus impairing embryo growth
and its ability to produce interferon γ (Sosa et al., 2006). High
plasma concentrations of insulin are associated with better
resumption of ovarian cyclicity in dairy cattle, but they also
interfere with oocyte competence resulting in low blastocyst
rate (Adamiak et al., 2005). The higher ovulation rate after
oestrus synchronisation in the nervous ewes might be asso-
ciated with the higher plasma concentrations of insulin, and
interference with oocyte competence could explain why it did
not result in similar prolificacy on day 30, as oocyte compe-
tence is a key factor in embryo survival (Krisher, 2004). The
differences in the timing of the reproductive losses between the
two lines may be explained as follows: the calm ewes possibly
lost their embryos late because of the negative relationship
between the number of ovulations and subsequent embryo
viability (Haresign, 1985), whereas nervous ewes may have had
difficulties either at conception (high concentrations of insulin
affecting oocyte competence) or during maternal recognition of
pregnancy (low concentrations of leptin affecting the quality of
the uterine environment).

The ewes of the selection lines have similar productive
outcomes when not challenged by the environment or
nutrition (Bickell et al., 2010a; Blache and Bickell, 2010;
Hawken et al., 2012). When under sub-optimal conditions,
the calm ewes perform better than the nervous ewes
(Hawken et al., 2012). These previous data have led us to
hypothesise that energy partitioning is different between the
lines, as nervous animals would have to spend more energy
on reacting to the sub-optimum environment or nutrition.
Reproduction responds to variation in nutrition and
abundant evidence exists of cross-talk between the repro-
ductive and metabolic axes (Blache et al., 2007). Although
BW and body condition score were not different between the
selection lines, insulin and leptin concentrations were,
suggesting that there is an energetic cost to having high
behavioural reactivity and this may involve differences in the
way energy is partitioned, and metabolic signalling may
affect the number of ovulatory follicles or the quality of the
oocytes. In fact, in numerous species from laboratory
rodents, companion animals to farm animals, domestication
and in a similar way selection for tameness or low level of
behavioural reactivity is associated with an increase in litter
size (Sethell, 1992; Price, 2002).

Conclusions

Behavioural reactivity affects ovulation rate but not the
occurrence of ovulation, so differences in reproductive out-
comes between the calm and nervous ewes were mainly due
to a higher ovulation rate in calm ewes. Some of the nervous
ewes, when the ovulation rate is maintained, have problems
in maintaining their pregnancy possibly because of the
quality of the oocytes and subsequent embryos, and perhaps
the quality of the uterine environment during the first
2 weeks of pregnancy. As plasma concentrations of progesterone post-AI were not affected by temperament, but insulin and leptin concentrations were, we suggest that reproduction in nervous ewes is compromised by factors leading up to ovulation and conception, or in the uterine environment during early pregnancy, that reflect differences in energy utilisation.

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References

Bickell SL, Poindron P, Nowak R, Ferguson DM and Blache D 2010b. Maternal behaviour at parturition in outdoor conditions differs only modestly between single bearing ewes selected for their calm or nervous temperament. Animal Production Science 50, 675–682.
Murphy PM 1999. Maternal behaviour and rearing ability of Merino ewes can be improved by strategic feed supplementation during late pregnancy and selection for calm temperament. PhD thesis, University of Western Australia, Perth, Australia.
Viñoles C, Paganoni B, Glover KMM, Milton JTB, Blache D, Blackberry MA and Martin GB 2010b. The use of a ‘first-wave’ model to study the effect of nutrition on ovine follicular dynamics and ovulation rate in the sheep. Reproduction 140, 865–874.