final report

Project code: AHW.085

Prepared by: Dominique Blache[1] & Drewe Ferguson[2]
[1]University of Western Australia
[2]CSIRO Livestock Industries

Date published: July 2009

ISBN: 9781741917109

PUBLISHED BY

Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Increasing Lamb Survival by Selection for Temperament

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

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Abstract

This research project was conducted to investigate the association between temperament, maternal behaviour and lamb survival. The research was conducted in industry meat and wool flocks (Sub-project A) and in the UWA temperament selection line flock (sub-project B). It was concluded that selection for temperament is not likely to alter maternal behaviour during and immediately subsequent to parturition. Therefore, as an on-farm strategy, selection for temperament is not likely to result in significant improvements in lamb survival. Small gains in colostrum quality and neonatal suckling behaviour/vigour, which are important to lamb survival, may arise but only in those flocks where there has been significant selection intensity for temperament over several generations.

Executive Summary

There is a need to improve lamb survival in sheep flocks particularly those produced under more extensive management systems. The level of lamb mortality that occurs typically within the first few days of life has been estimated at 10 – 15% but it is not uncommon for higher losses to occur. For the Australian sheep industry, the cost of lamb losses (birth to weaning) has been estimated at 10 million per year. Apart from the economic impact, the high level of lamb mortality represents a significant animal welfare issue.

The causal factors associated with lamb mortality include climatic conditions, starvation, exposure, infection, physiological condition of the ewe and dystocia. Some of these factors arise through the absence or late establishment of the vital ewe-lamb bond that must occur within the first few hours after birth. The expression of maternal behaviour that is important during the establishment of this bond can be influenced by the intrinsic fearfulness or temperament of the ewe. Temperament has been shown to be a moderately heritable trait (h^2 0.25 – 0.5) and a simple on-farm test known as the isolation box test (IBT) has been developed. Therefore selection for temperament may provide an indirect means for improving lamb survival. The primary goal of this project was to test this hypothesis by exploring the associations between temperament, maternal behaviour and lamb survival.

The objectives of the project were as follows:

1. To determine whether selection for temperament will improve reproductive efficiency and neonatal lamb survival in commercial wool and meat sheep flocks.

2. To investigate the interaction between genetic and environmental factors on the establishment of the ewe-lamb bond and subsequent lamb survival.

3. To develop improved on-farm management strategies (based on the outcomes of objectives 1 and 2) that focus on managing the pregnant ewe and increasing lamb survival.

4. To train 2 PhD students in the field of farm animal welfare.

The project comprised two research sub-projects. Sub-project A was focused on quantifying the benefits of selection for temperament on lamb survival in commercial flocks and sub-project B investigated how maternal behaviour and physiology was influenced by genetic and environmental factors.

In stage 1 of Sub-project A, lamb survival was studied in seven industry flocks (4 Merino and 3 terminal sire meat flocks – total of 5791 ewes) where ewes assessed as either good and poor temperament were joined to sires with low (good temperament) and high (poor temperament) EBVs for IBT agitation score, respectively. In Stage 2 of this sub-project, the F1 Merino ewes (n = 545) which were genetically different for temperament were joined to further examine the association between temperament and lamb survival under industry conditions.

In summary, temperament was not found to significantly influence lamb survival in the commercial flocks (Stages 1 and 2). Whilst it is possible that far greater selection pressure for temperament over several generations would be required before an effect on maternal behaviour and/or lamb survival was observed, the results from the UWA selection line flock (sub-project B) suggest that such associations may still be inconsistent.

In sub-project B four specific investigations were undertaken over 3 years using Merino ewes divergently selected for temperament from the UWA temperament selection line flock. These examined the:

- Interaction between genotype and maternal behaviours on expression of temperament.
- Effect of social isolation and temperament on maternal behaviour.
- Effect of temperament on ewe-lamb discrimination in the face of novel stressors.
- Effect of temperament on maternal behaviour expressed during parturition and bonding.

The key findings from sub-project B were:

- Expression of temperament is genetically controlled rather than being a behaviour learned from early interactions with the ewe.
- Several behavioural factors between the ewe and lamb thought to be relevant to the differences in lamb survival between the temperament selection lines were not found to be important.
- Nervous ewes produced thicker colostrum and their lambs took more time to find the udder. These factors may result in reduced or retarded supply of nutrients to the lamb and consequently, will have a bearing on its survival.

Based on the combined results from the two sub-projects, it is concluded that selection for temperament is not likely to alter maternal behaviour during and immediately subsequent to parturition. Therefore, we conclude that as an on-farm strategy, selection for temperament will not result in significant improvements in lamb survival. Small gains in colostrum quality and neonatal suckling behaviour/vigour and therefore, lamb survival may arise but only in those flocks where there has been significant selection intensity for temperament over several generations.

It is our recommendation that whilst there is scope for genetic improvement in reproductive traits like lamb survival, greater gains will be achieved through increased adoption of existing knowledge and strategies centred on improved nutrition and management of the gestational and post-partum ewe.

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1 Background

In the quest for increased on-farm profitability, the Australian sheep industries are confronted with a myriad of challenges. It is now well recognised that improvements in productivity and efficiency cannot come at the expense of the environment or animal welfare. With respect to the latter, it is vital that our sheep production systems remain proactive with respect to improving animal welfare in order to maintain and indeed increase consumer confidence in its products. Historically, the issue of lamb mortality has generally been considered more in terms of production losses. However, this issue clearly has a significant animal welfare dimension given the likely negative public perceptions to massive lamb losses that can occur. From Australian and New Zealand data, it is estimated that neonatal losses of lambs is in the order of 10% to 15% (Alexander 1983, Matthews 1996). For the Australian sheep industry, the cost of lamb losses (birth to weaning) has been estimated at 10 million per year (conservative estimate calculated from Walker et al. 2003). The causal factors for these losses include climatic conditions, starvation, exposure, infection, physiological condition of the ewe and dystocia (Hight and Jury 1970). Some of these factors arise through the absence or late establishment of the vital ewe-lamb bond that must occur within the first few hours after birth (Nowak, et al. 2004). The importance of the rapid establishment of the ewe-lamb bond is reflected in the fact that the highest proportion of lambs died (50-60%) within the first 3 days of life (Nowak, et al. 2000).

The establishment of a strong ewe-lamb bond during the first hours after birth has been intensively studied and several factors have been identified as crucial to this process (for review see Nowak et al 2004). Ewes, at lambing, seek isolation and shelter, which is, for a gregarious species, a potential source of stress during and immediately subsequent to birth. In the Merino ewe, this stress is mainly due to the conflict between care of the lamb and maintaining contact with the flock (Stevens, et al. 1982). Once the lamb is expelled, the ewe stands up and licks the newborn. The duration of grooming of the newborn is essential to the establishment of the bond and can be reduced in ewes that are primiparous or that have experienced difficult births. Most of the grooming occurs during the first hour after parturition. During this very short window, the lamb and the mother learn each others signatures which are essential for future recognition. Olfaction and behaviour are the main cues used by the mother whilst visual and auditory cues are more important for the lamb. Being a learning experience, it is essential that both ewe and lamb are not disturbed during this first hour. The expression of maternal behaviour is controlled by many hormonal factors acting before (sex steroids), during and after parturition (Oxytocin, opioids, corticotrophin-releasing factors; Poindron and Le Neindre 1980). In addition, the intrinsic emotional reactivity (ER) or temperament of the ewe will also significantly influence the quality of the maternal behaviour. Nutritional factors also influence the strength of the ewe-lamb bond. For example, low levels of colostrum production and low quality colostrum will decrease the vigour and health of the lamb (Nowak, et al. 2004).

In summary, emotional, nutritional or physical stressors can jeopardise the formation of a strong bond between the ewe and the lamb and this can inevitably affect the survival of the new-born lamb (Nowak, et al. 2004). Strategies focused on pregnant ewe nutrition and management have been developed and have been successfully applied on-farm to increase lamb survival. However, there is scope to further decrease lamb mortality and neonatal suffering by decreasing the general reactivity to stress through selection for improved temperament.

Temperament, which is defined as the animal's emotional reactivity to humans and novel or threatening environments, can be measured using a range of escape/avoidance tests. Over the last 18 years, the UWA Animal Biology department has applied these tests to establish two experimental lines of Merino sheep – "calm" and "nervous". A key outcome of the research using this unique resource was that calm ewes are clearly better mothers than nervous ewes as they spend more time with their lambs, have a shorter flight distance when disturbed and return to their lambs faster than nervous ewes (Murphy, et al. 1994; Murphy 1999). Consequently, lamb mortality for calm ewes was approximately 50% lower than that of nervous ewes, for both single and twin bearers. Clearly, these results are very promising however, the impact of selecting for temperament on lamb survival needs to be tested under commercial on-farm conditions.

A simple reliable objective test for the on-farm measurement of temperament in sheep has now been developed (MLA Project SHGEN.025). The isolation box test (IBT) was found to be repeatable and agitation score, the trait measured during the IBT, was found to be moderately heritable (h^2 0.25 – 0.5) in 4 breeds (Merino, Border Leicester, Poll Dorset and White Suffolk). The purpose of this project is to apply this test on-farm to demonstrate that selection for temperament can yield positive reductions in reproductive loss and neonatal lamb suffering and mortality in commercial flocks. Secondly, the project will develop new knowledge with respect to the genetic and environmental regulation of the establishment of the ewe-lamb bond. The project outcomes and new knowledge will be applied to augment existing management strategies to further improve lamb survival.

2 Project Objectives

1. To determine whether selection for temperament will improve reproductive efficiency and neonatal lamb survival in commercial wool and meat sheep flocks.

2. To investigate the interaction between genetic and environmental factors on the establishment of the ewe-lamb bond and subsequent lamb survival.

3. To develop improved on-farm management strategies (based on the outcomes of objectives 1 and 2) that focus on managing the pregnant ewe and increasing lamb survival.

4. To train 2 PhD students in the field of farm animal welfare.

3 Methodology

The project comprises two research sub-projects. Sub-project A is focused on quantifying the benefits of selection for temperament on lamb survival in industry flocks (Objective 1) and sub-project B investigated how maternal behaviour and physiology can be influenced by genetic and environmental factors (Objective 2).

3.1 Sub-project A – Selection for temperament and lamb survival on-farm

3.1.1 Stage 1

Collaboration with seven commercial flocks comprising four wool (*Merinotech* WA, *Grindon* WA, *Billandri* WA and *Washpool*, SA) and three prime lamb flocks (*Genstock*, WA, *Gnaring Park*, WA and *Eagleridge,* VIC) was obtained. Approximately 800-1000 ewes/flock were used in the experiment (Total = 6200 ewes). Of these, a group of approximately 200-300 ewes were randomly drafted off as the control group. The remainder were tested for temperament using the isolation box test (IBT) and the 200-300 ewes with the lowest and highest IBT scores were selected as the good and poor temperament groups, respectively. Blood samples (6ml EDTA vacutainers) were collected from these ewes by jugular venipuncture for subsequent DNA parentage assignment of their lambs.

A total of 5791 ewes were subsequently artificially inseminated to selected sires. An associative joining program was used where ewes from the good temperament group were joined to sires with low EBVs for IBT agitation score (ie. good temperament). Similarly, sires with high EBVs for the trait (ie. poor temperament) were used in the poor temperament ewe group. The sire EBVs for IBT agitation score are shown in Table 1.

Ewes from the control group were artificially inseminated (AI) with semen from industry sires (3 Merino and 2 Poll Dorset) which had no progeny data on temperament. These sires also served as link sires across the flocks within each production system (wool and prime lamb).

The ewes were pregnancy scanned 60 days after AI. After drafting off the non-pregnant ewes or ewes assessed as pregnant to back-up rams, the remaining ewes were separated into two groups based on scanned litter size (single and multiple bearing ewes). These two groups, which included all three temperament treatments (Good, Poor and Control) were managed separately.

During gestation through to lambing, basic information on the pasture and climate conditions was captured. During gestation, it was necessary to provide supplements to the ewes on all seven collaborating farms. The type and amount of supplement offered was recorded.

During the lambing phase (Day 145 – 155 after AI), human contact with the ewes was kept to a minimum. The lambing paddocks were monitored daily and tissue samples from lambs found dead were collected for DNA extraction.

Table 1 Sire EBVs for IBT agitation score within breed.

Breed	Sire ID	IBT Agitation Score EBV	Temperament Group
Merino	5003831999995873	-30.51	Good
	5030542001010228	-38.41	Good
	5030702002020150	-30.02	Good
	6012502001107439	-35.65	Good
	6012502001107448	-42.90	Good
	50158719999J665	-48.60	Good
	5047671999OR0382	-28.03	Good
	5030541997970186	63.72	Poor
	5049011999990075	18.87	Poor
	6005711999990354	38.41	Poor
	6009992000000189	32.91	Poor
	6010822000001217	38.46	Poor
	6012502001107351	51.82	Poor
	5047672001VI0172	38.01	Poor
Poll Dorset	1601852003030236	-20.57	Good
	1622882002020099	-25.33	Good
	1640732002020433	-32.98	Good
	1618922002020555	26.81	Poor
	1623682001010351	13.63	Poor
	1640732002020284	34.57	Poor
White Suffolk	2300262001012020	-30.24	Good
	2300992003030135	-34.34	Good
	2301132002020124	20.72	Poor
	2301222001011131	30.82	Poor

At marking, the lambs were ear tagged, weighed and a sample of blood (6ml EDTA vacutainer by jugular venipuncture) was collected for parentage assignment. The lambs were returned to the ewes until they were weaned. Within two weeks after weaning, the IBT agitation score and liveweight of the lambs was recorded.

The live weight of the ewes was recorded at three time points, during temperament screening, pregnancy scanning and at lamb marking.

Parentage assignment
DNA was extracted using QIAGEN DNeasy Blood and Tissue kits according to the procedures of the manufacturer.

A sample of blood from each ewe and lamb was transferred onto cards from the Catapult® test kits. These cards plus semen from all 29 rams and extracted DNA (dead lambs) were sent to Catapult Genetics for parentage assignment of all lambs.

Statistical analysis
After collating the data and identifying records where the parentage of the lambs could not be assigned, the database was created based on the scanned records. Each lamb identified at scanning was given a unique identification. A further 146 records were added to the database which represented additional lambs that were not detected at scanning but were identified through DNA parentage assignment. These lambs were typically from ewes where the litter size had been underestimated by the scanner.

Within the database, lamb survival was defined as a binomial trait where each record was assigned either 0 (dead or missing) or 1 (alive at marking).

For the analysis of lamb survival, the mixed model procedure in SAS (SAS 1999) was used where flock, temperament group and litter size were included as fixed effects and sire was included as a random effect. Separate analyses were performed for the wool and meat flocks. The model used to analyse the trait IBT agitation score in the live progeny included the fixed effects of flock, temperament group, sex and the random term sire. The covariate weaner liveweight was also fitted in this model.

3.1.2 Stage 2 – F1 Merino ewes

Following on from Stage 1, the effect of temperament on reproductive losses in F1 ewes (varying in temperament) from the commercial Merino flocks was quantified.

(i) WA Flock (*Cathair Donal*)
The F1 ewes from the three WA flocks (*Merinotech, Grindon* and *Billandri*) were transferred and grouped together on another WA property *Cathair Donal*. Only the F1 ewes from the good and poor temperament groups were used totalling 393 ewes.

The ewes were weighed and fat scored (AUS-MEAT fat scoring system 1 - 5 scale) prior to AI. Thirty-seven ewes were excluded from AI because their fat score was below the agreed minimum threshold of 2. At the time of AI, a further 63 ewes that originated from *Merinotech* were excluded because the AI operator considered that they were already pregnant. This was subsequently confirmed at pregnancy scanning where 52 ewes were diagnosed pregnant approximately 1 month before AI. This subset were drafted off after scanning and run as a separate mob and a decision was made to record lambing data on this group.

A total of 273 ewes were artificially inseminated and managed as a group throughout their pregnancy. The ewes were scanned for pregnancy status and litter size 73 days after AI.

During gestation, the pasture conditions were assessed and herbage mass was estimated. The decision to supplement ewes was based on these estimates and the condition of the ewes.

Approximately 3 weeks prior to lambing, large neck tags were put on the ewes to assist in visual identification during lambing. Lambing observations (3 times/day) commenced on day 148 of gestation. For each live lamb, the following procedures and measurements were conducted:

- Ewe identification
- Date and estimated time of lambing
- Paddock
- Approach distance (estimated minimum distance before ewe left birth site or lamb on approach by human)
- Distance from birth site/lamb (estimated maximum distance the ewe retired to during the lamb tagging and measurement)
- Time to return (time the ewe took to return to birth site or lamb after departure of human)

- Eartag lamb
- Lamb sex and weight
- Litter size
- Lambing ease (where possible)

Ewes were not approached within an estimated 2 h from birth to allow for the ewe-lamb bond to occur. If a ewe was having difficulty lambing, assistance was provided approximately 3 h after initial observation.

On discovery of a dead lamb, the following measurements were recorded and the lamb was autopsied to identify cause of death.

- Date and time
- Paddock
- Sex
- Weight
- Lamb eartag (where possible)
- Dam if known

When the lambs were 4-6 weeks of age they were marked. The ewes were weighed and body condition and udder scores were assessed and this was repeated at weaning. The temperament of the lambs was determined using the IBT one week after weaning according to the protocols of Blache and Ferguson (2006).

(ii) SA flock (*Washpool*)

The ewes were weighed and fat scored and a total of 152 ewes were accepted for AI. They were subsequently scanned for pregnancy status and litter size 62 days after AI.

The same procedures described above (gestation to weaning) were applied in the SA flock.

Statistical analysis

Separate analyses for each flock were undertaken. Ewe behaviour (approach distance, distance from birthsite/lamb and time to return to birthsite/lamb) were analysed used Proc GLM in SAS (SAS 1999) where temperament group and litter size were included as fixed effects. For the analysis of lamb survival, the mixed model procedure in SAS was used where flock of origin (*Cathair Donal* flock only), temperament group and litter size were included as fixed effects and sire was included as a random effect. The model used to analyse the trait IBT agitation score in the weaner progeny included the fixed effects of flock of origin (*Cathair Donal* flock only), temperament group, sex and sire as a random term. The covariate weaner liveweight was also fitted in this model.

3.2 Sub-project B – Influence of genetic and environmental factors on maternal behaviour and physiology.

This sub-project investigated the interaction between genetic and environmental factors on the establishment of the ewe-lamb bond and subsequent lamb survival.

Four specific investigations were undertaken over 3 years using Merino ewes divergently selected for temperament from the UWA temperament selection line flock. Details regarding the establishment of the lines and the selection procedure were reported by Blache & Ferguson (2005). Briefly, Merino ewes have been selected, over 18 generations, for calm (C) or nervous (N) temperament using an arena test and the isolation box test. Since their establishment, the two lines have been maintained as a single flock, except during joining where they were separated into sire groups with 5-7 rams.

3.2.1 B.1 Interaction between genotype and maternal behaviours on expression of temperament

We investigated the relative contributions of genotype versus the post-partum behaviour of the dam on the temperament of the lambs using a cross fostering procedure.

A detailed description of the materials and methods is provided in the manuscript in Appendix 9.2 Genotype rather than non-genetic behavioural transmission determines the temperament of Merino lambs (accepted in *Animal Welfare*). Briefly, 48 multiparous calm (C) and 52 nervous (N) ewes were artificially inseminated with semen of a sire of the same temperament. The ewes were brought indoors in single pens prior to lambing and parturition was induced via a dexamethasone injection. At birth, 32 lambs of a given temperament line were cross fostered to ewes from the other line (16 N to C, 16 C to N), 34 lambs were cross fostered to ewes from the same line (15 C to C, 19 N to N) and 30 lambs were left with their birth mother (15 C, 15 N), to control for the effect of cross fostering. The temperament of the lambs was assessed at two occasions, 1 week after birth by measuring locomotor activity during an open field test and at weaning (16 weeks) by measuring locomotor activity during an arena test and agitation score measured during an isolation box test.

3.2.2 B.2 Effect of social isolation and temperament on maternal behaviour

B.2a
It is known that temperament influences maternal behaviour and lamb survival in Merino sheep selected for calm or nervous temperament. The impact of this selection on mother-young recognition and early expression of temperament in lambs is unknown. In this experiment, we tested the ability of multiparous ewes selected for calm (n = 16) or nervous (n = 18) temperament to recognise their own lambs 6 h after parturition, the ability of the lambs to display a preference for their own mother at 18 h, and the temperament of the lambs at 1 and 16 weeks of age. The detailed description of the materials and methods is provided in the published paper in Appendix 9.3 Bickell et al (2009) Temperament does not affect the overall establishment of mutual preference between the mother and her young in sheep measured in a choice test. *Developmental Psychobiology 51:428-438*).

B.2b
The aims of this study was to examine whether maternal behaviour between ewes selected for a calm or nervous temperament is independent of environmental conditions and to determine if the level of maternal behaviour is correlated with the concentration of oestradiol, progesterone and cortisol during the peripartum period. These hormones are known regulators of maternal behaviour. Oestradiol, progesterone and cortisol concentrations from 4 days before parturition through to 24 hours after parturition were determined from blood samples collected from 10 calm

and 12 nervous ewes. Behavioural interactions between ewe and lamb were also recorded for 2 hours starting at parturition. The methodology is described in the draft journal manuscript attached as Appendix 9.4: Maternal behaviour and peripartum levels of oestradiol, progesterone and prolactin show little differences in Merino ewes selected for calm or nervous temperament under indoor housing conditions. (Submitted to the journal *Animal*).

B.2c
Colostrum provides essential nutrition to neonatal lambs and is essential to lamb survival. In this study the aim was to determine whether ewe temperament affects the quantity and quality of colostrum that ewes produce. Colostrum was collected at birth, 1, 3, 6 and 10 h post-partum in 15 and 19 ewes from the calm and nervous lines, respectively. It was subjectively assessed for viscosity and analysed for fat, protein and lactose composition. The complete description of the methodology is provided in the journal paper that was published by Hart et al (2006) attached as Appendix 9.5 Colostrum quality of ewes of calm temperament is not responsible for low mortality. *Aust. J. Exper. Agric.* **46**:827-829.

3.2.3 B.3 Effect of temperament on ewe-lamb discrimination in the face of novel stressors

Novelty is known to be a powerful fear inducing stimulus and thus exposure to novelty can be a stressful experience for an animal. During lambing, the presence of a disturbing stimulus could have detrimental effects on the capacity of ewe and the lamb to recognise each other because stress could impair their learning capacity. The ability of ewes and lambs to show a preference for each other was tested at 6 and 18 hours after parturition in a two-choice discrimination test involving a novel and disturbing object in order to detect potential differences between the two temperament lines in this challenging situation.

The methodology is described in more detail in the draft journal manuscript attached as Appendix 9.6: Challenge by a novel object does not impair the capacity of ewes and lambs selected for a nervous temperament to display early preference for each other. (Submitted to the journal *Developmental Psychobiology*).

3.2.4 B.4 Effect of temperament on maternal behaviour expressed during parturition and bonding

The aim of this study was to investigate if the selection for temperament influenced the behaviour of the ewe and lamb after parturition. Over three years, 326 ewes from the calm line and 282 ewes from the nervous lines were synchronised in oestrus and naturally mated and deemed pregnant by ultrasound scanning. Ewes were aged between 2 – 7 years and were primiparous (maiden) and multiparous (experienced). Ewes were side branded with stock-marker spray to facilitate identification from a distance.

Lambing took place in four paddocks at the University of Western Australia's research farm at Wundowie, located approximately 60 km north-east of Perth (latitude 31˚ 46' south, longitude 116˚ 29' east; elevation of 330 m above sea level). The paddocks were divided into night and day paddocks. Pregnant ewes were moved from the day paddock to the night paddock at approximately 17:00, and from the night paddock to the day paddock at approximately 07:00. Ewes that had lambed in the day paddock the previous day were moved out of the paddock at approximately 07:00 the next morning, while ewes that lambed in the night paddock were moved out of the paddock at approximately 17:00 in the evening. Lambs born during the day were ear tagged in the evening while lambs born during the night were ear tagged in the morning.

Lambing procedure
Ewes lambed without assistance as far as possible. However, if the ewe had begun labour but had not delivered the lamb after it had been visible for approximately two hours then assistance was given.

Behavioural observations
The lambing flock was observed from a distance by trained observers sitting in an observation tower in the day paddock for a period of approximately four weeks during daylight hours (08:00-17:00). Remote controlled recording devices (RCRD) were used to video record the behavioural interactions between the ewe and her lamb for up to two hours postpartum. The ewes were habituated to the RCRD for two weeks before parturition. Once a ewe was observed to be in advanced labour (feet visible) the RCRD was moved into position, approximately 2-4 m away from the ewe. In addition, behavioural interactions between ewe and lamb were recorded for 2 hours starting at parturition by the observers in the tower. The duration of labour was recorded from the first appearance of the waterbag until the birth of the lamb. The behaviours recorded by observer or later extracted from the RCRD footage are listed in Table 2. The number, temperament and parity of ewes giving birth during the day or night were also recorded. Observations made on twin lambs were not retained for analysis due to the small sample size. Due to recording difficulties not all data was available for each animal and some records do not extend for the whole two hours postpartum. The time at which the ewe moved > 3 m away from the birth site was calculated using Rangefinder binoculars and a compass. The ewe’s movements from the birth site were observed during a preliminary study conducted during 2006, however, the ewe’s movements from the birth site could not be recorded during 2007 and 2008, due to the intensity of the lambing observations.

Table 2 Ewe and lamb behaviour quantified form live observation of recorded observation

Maternal Behaviour	Description
Licking	Licking of the lamb.
Low pitched bleat	A low pitched 'rumble' or 'mmm' bleat made with the mouth closed.
High pitched bleat	Louder 'baa' bleat made with the mouth open.
Terminating sucking bout	Ewe moves away from the lamb while lamb is at the udder
Circling	Ewe steps sideways, moving hindquarters away from the lamb when it goes toward the inguinal region.
Backing	Ewe steps backwards as lamb moves forwards toward the inguinal region.
Head butt	Ewe’s head makes contact with the lamb following a fast movement of head
Head threat	Ewe makes a fast movement of head as if to head butt lamb, but does not make contact
Lamb behaviour	**Description**
Suckles at the udder	Lamb has teat in its mouth for at least 5 s.
To knees	Lamb on chest, pushes up on knees, supporting part of body off the ground.
Attempts to stand	Lambs stands on all 4 feet for less than 5 s.
Stands	Lamb stands on all 4 feet for more than 5 s.

Statistical analyses

Ewe and lamb behaviours were compared between calm and nervous animals using a two factorial (temperament, experience) analysis of variance (ANOVA) using year as a blocking term with Genstat Eighth Edition (VSN International Ltd, Hemel Hempstead, United Kingdom). A square root transformation was used when data was not normally distributed. The time ewes spent licking, grazing and looking up was analysed with a repeated measures two factorial ANOVA. The proportions of calm and nervous ewes giving birth during the day or night were compared with a Chi Square test. The distance the ewe moved from the birth site every hour was calculated using the distance between the tower to the birth site (BS), the distance between the tower to the position of the ewe (EWE) and the angle between these to positions (ANGLE) according to the equation =SQRT($BS^2 + EWE^2$ – (2 x BS x EWE x cos (radians(ANGLE)))). The differences between temperament and experience in movements from the birth site were analysed with a two factorial ANOVA. The effect of temperament, experience and year on the mortality rate of lambs from birth to weaning (16 weeks) was determined using a Chi Square test.

4 Results and Discussion

4.1 Sub-project A – Selection for temperament and lamb survival on-farm

4.1.1 Stage 1

The results from Stage 1 were presented in earlier milestone report and this is attached as Appendix 9.1. For the purposes of this final report only a summary is presented in the following with the primary focus being on the stage 2 results.

For the seven flocks the pregnancy rate (No. of foetuses/pregnant ewe %) was moderately high ranging from 126.9 – 160.1% for Merinos and 146.3 -167.4% for Terminal sire flocks. This was attributed to higher multiple pregnancies. Lamb survival was significantly influenced by flock ($P<0.001$) and litter size ($P<0.001$) but not temperament group. Lamb survival (No. of lambs at marking/No. of expected lambs %) ranged from 35.5 – 81.2 % and 51.7 – 71.7% for the Merino and Terminal sire flocks, respectively. The results for each flock were: *Billandri* (wool WA) 46.9%, *Merintech* (wool WA) 73.1%, *Grindon* (wool WA) 35.5%, *Washpool* (wool SA) 81.2, *Genstock* (teminal WA) 61.1%, *Gnaring Park* (terminal WA) 51.7% and *Eagleridge* (terminal VIC) 71.7%. Overall, lamb survival was quite low in most of the WA flocks (Merino and Terminal sire) and this was directly linked to the poor pasture conditions during gestation despite supplementary feeding in some cases. There was a commensurate decrease in lamb survival with an increase in litter size. The IBT agitation score of the progeny was significantly affected by flock ($P<0.001$ Merino and Terminal sire), temperament group ($P<0.05$ Merino; $P=0.06$ Terminal sire) and sex ($P<0.01$ Merino; $P<0.001$ Terminal sire). A significant interaction between flock x temperament group ($P<0.05$) was also observed for the Merino flocks. Importantly, genetic divergence in temperament was evident in the Merino F1 progeny which was critical as these ewes will be joined in 2008 (Stage 2) to further test the hypothesis that temperament affects maternal behaviour and this in turn, will have a significant effect on lamb survival.

4.1.2 Stage 2 – F1 Merino ewes

Ewe liveweight and condition score
The change in mean ewe liveweight and condition score in both flocks is shown in Figure 1. The condition score of the *Washpool* ewes declined slightly from AI until the end of March but then trended upwards right through to weaning. These trends align with the pasture conditions and supplementation regime. In the WA flock, ewe body condition score declined during May and June when the pasture conditions were at their poorest and despite provision of grain and hay supplementation. Condition score fell just below 2.5 and essentially remained at this level right through to weaning.

The pasture conditions during late pregnancy were good with 2880 and 1999 kg DM/ha of herbage mass (food on offer - FOO) for the SA and WA flocks, respectively. From research undertaken within the Lifetime Wool project, the lowest lamb mortality (up to 48 h post-partum) was observed when FOO ranged between 1500 and 2000 kg DM/ha (Ferguson et al 2004; Oldham et al 2005).

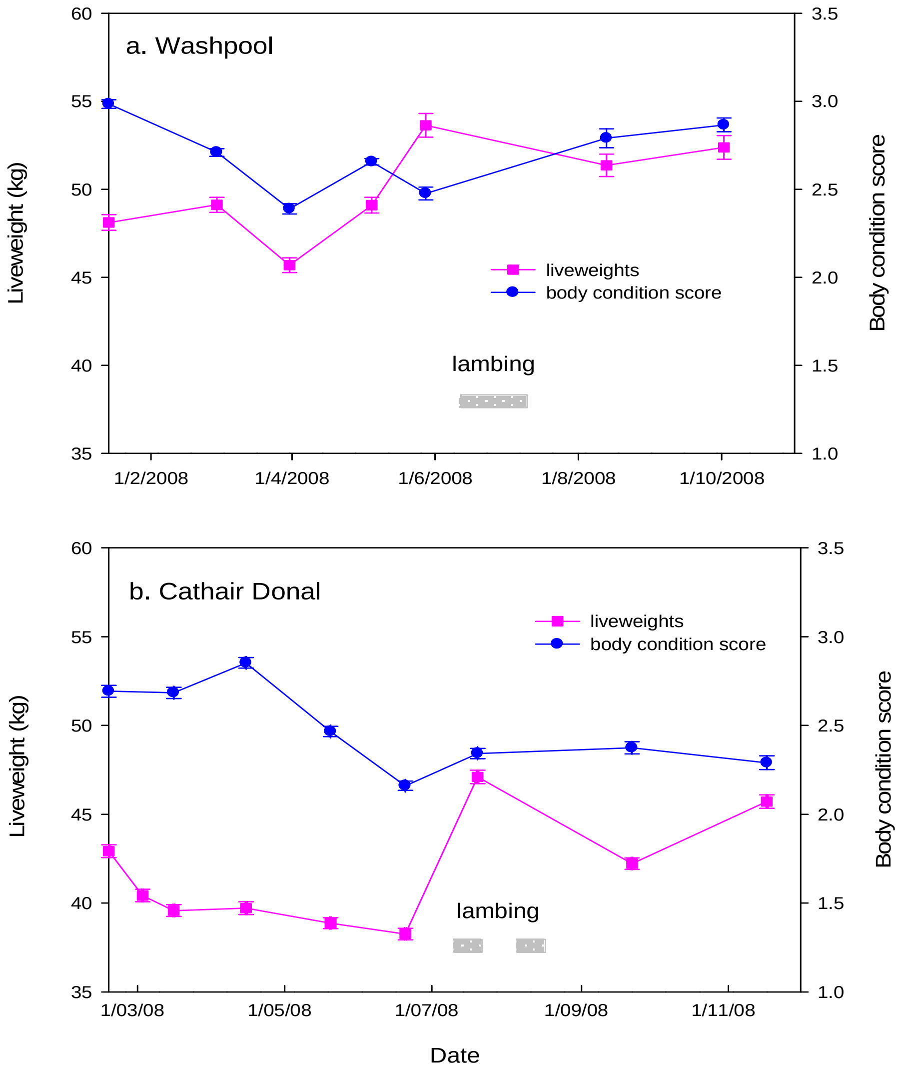

Figure 1 Changes in mean ewe liveweight and condition score from AI to weaning in the two flocks

A summary of the reproductive and lambing results for the AI ewes from the two flocks is presented in Table 3. A similar summary of the lambing results for the early pregnancy ewes from *Cathair Donal* is presented in Table 4.

Table 3 Summary of the reproductive and lambing results for the two flocks.

	Flock	
	Washpool	***Cathair Donal***
Dates		
AI	16/1/08	18/3/08
Pregnancy scanning	31/3/08	20/5/08
Lambing	11 – 20/6/09	13 – 21/8/08
Marking	13/08/08	22/9/08
Weaning	20/9/08	11/11/08
Pregnancy		
Number of ewes AI'd	152	273
Number of ewes pregnant	84	175
Number of ewes with 2 foetuses	14	19
Number of ewes with 1 foetus	70	156
Number of foetuses scanned	98	194
% ewes pregnant	**55.3**	**64.1**
% foetuses/ewe joined	**64.5**	**71.1**
% foetuses/ewe pregnant	**116.7**	**110.8**
No. of additional lambs (not identified at scanning)	2	2
Expected total lambs (includes additional)	**100**	**196**
Lambing		
Number of lambs tagged at lambing (live and dead)	93	193
Number of live lambs at lambing#	85	167
• Single	62	137
• Twin	22	27
• unknown	1	3
Number of dead lambs at lambing#	8	26
• singles	6	15
• twins	0	5
• unknown	2	6
Number of lambs with unknown or ambiguous pedigree	2	14
Number of empty ewes (scanned pregnant)	1	3
Number of expected lambs from empty ewes	1	4
% live lambs at lambing / expected lambs	**86.7**	**86.1**
% live lambs at lambing / ewes joined	**55.9**	**61.2**
% live lambs at lambing / ewes pregnant	**101.2**	**95.4**
Marking & Weaning		
Number lambs at marking	80	156
Number lambs at weaning	75	150
% live lambs at weaning / expected lambs	**75.0**	**79.6**
% live lambs at weaning / live lambs at lambing	**86.2**	**82.0**

lambing period (9-10 days) identified in the table.

Table 4 Summary of the lambing results for the ewes from the early pregnancy group (*Cathair Donal*).

Lambing	***Cathair Donal*** 9/7/08 to 16/7/08
No of ewes identified as pregnant prior to AI	51
Number of lambs tagged	62
Number live lambs at lambing	52
Number dead lambs at lambing	10
Lambs present at marking	54
Lambs present at weaning	53

Conception rate
The conception rates ranged from 55.3 % to 66.1% which is reasonably high given these were maiden ewes. Fifty percent conception would be a common industry average in maiden Merino ewes following AI (Jen Smith *pers. comm.*). There were no significant differences in pregnancy rate due to temperament group (*Washpool* – Good 54.5 %, Poor 55.8%; *Cathair Donal* - Good 67.4%, Poor 59.6%).

There were 5 ewes in total from both flocks (1 *Washpool*; 4 *Cathair Donal*) that did not lamb which could have been due to a misdiagnosis at pregnancy scanning or abortion in the latter half of gestation. Only one ewe died during lambing and she was from the early pregnancy group at *Cathair Donal*.

Lamb survival
Perhaps the best benchmark of lamb survival is the number of live lambs at lambing as a percentage of the total number of expected lambs based on scanning. The lamb mortality over the lambing period in both flocks (AI ewes) was relatively low 13 – 14% considering these were maiden ewes. These results are similar or better than other published estimates in Merino flocks (64-82% Kilgour (1992), 72.5% Kleeman and Walker (2005)). The low lamb mortality can be attributed, in part, to the good condition of the ewes at lambing (Figure 1) and the fact that extreme winter conditions were not encountered during lambing in both flocks (Figure 2). However, the lamb mortality appears higher in the early pregnancy group at *Cathair Donal* (Table 4). This group of ewes were not scanned for litter size so the expected number of lambs at birth was not known. The percentage of live lambs/total lambs at birth was marginally lower for this group (84%) compared with the AI group (86.5%).

The lamb losses from lambing through marking and weaning were also relatively low Table 3).

The results from the post-mortem analysis of lambs that died during the lambing period revealed no obvious trend with respect to the prevalent cause of death (Table 5). The data was not amenable to statistical analysis given the low numbers. The lambs that died *in utero* or during the process of parturition (not viable at birth) were low (n = 3). The total numbers of lambs that died due to either dystocia (n=11), starvation/exposure (n=11) or unknown causes (n=9) were similar. Of the lambs that died due to dytocia, all except one unknown litter size lamb were singletons with high birth weights (*Washpool* 4.7 – 6.0 kg; *Cathair Donal* 5.75 - 6.8 kg). The majority of lambs (>75%) died within the first three days after birth which is consistent with the literature (eg. Alexander 1983).

Table 5 Post-mortem analysis of the deaths during lambing.

Flock	**Cause of death**			
	Not viable at birth	Dystocia	Starvation/exposure	Unknown
Washpool				
Good temperament				
Singles	0	0	0	1
Twins	0	0	0	0
unknown	1	0	0	0
Poor tempperament				
Singles	0	3	1	0
Twins	0	0	0	0
unknown	0	1	1	0
Cathair Donal				
Good temperament				
Singles	0	3	1	2
Twins	0	0	2	0
unknown	2	0	2	1
Poor tempperament				
Singles	0	4	2	3
Twins	0	0	1	2
unknown	0	0	1	0

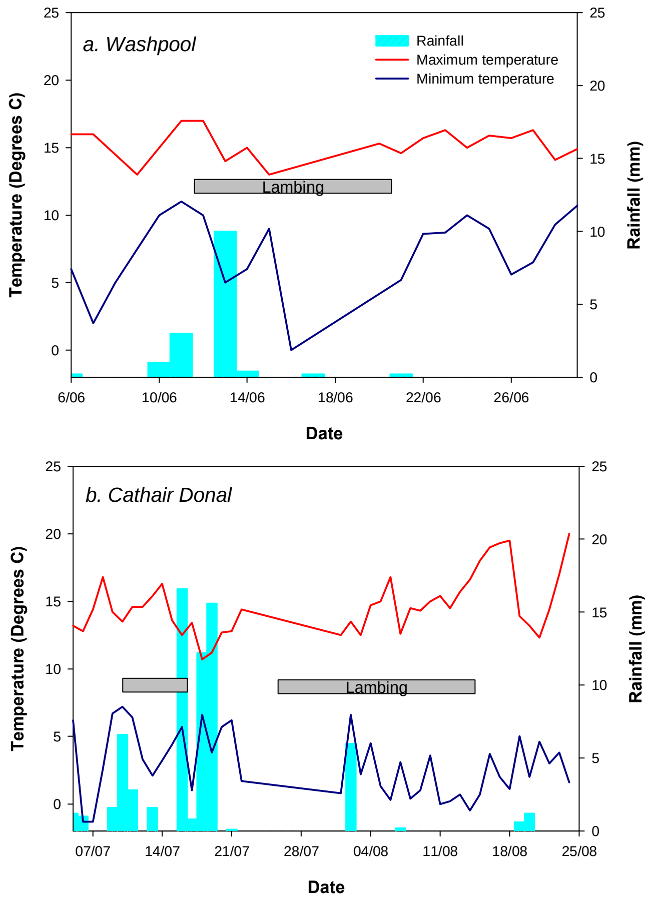

Figure 2 Minimum and maximum temperatures and rainfall during the lambing periods in both flocks.

There was no significant difference in lamb survival between the temperament groups in either flock. Our initial hypothesis was that mortality would be higher in the poor temperament group and that there would be a higher proportion of deaths due to starvation/exposure in this group. This was based on the view that the expression of maternal behaviour would be affected by temperament and the poor temperament ewes would be more easily disturbed and be less attentive to their lambs. However, this was not observed. Notwithstanding the fact that the overall level of lamb mortality was low, the lack of any temperament effect could be due to a lack of genetic divergence in the trait given the selection only applied to one generation.

However, the results from field observations of the UWA temperament selection lines are also relevant in this context (refer section 4.2.4). Under natural, undisturbed lambing conditions calm (good temperament) and nervous (poor temperament) ewes did not differ greatly in their maternal behaviours. These more recent results did not align with those of Murphy (1999), and the latter was used as the basis for our initial hypothesis. Differences in the time of tagging, and consequently differences in the timing of the first contact with human, could explain these discrepancies, because Murphy tagged her lamb during the observation, when we tagged then after the ewe and lamb behaviours were observed. A similar difference was also found during the study conducted with the Allandale flock (section 4.2.3 and appendix 9.6) Apart from the minor behavioural differences between the selection lines, lamb mortality only differed in 2008 but not 2007. In the 2008 lamb mortality was higher in the nervous line (18%) compared with the calm line (11%).

Ewe behaviour post-partum
Three behaviours of the ewe were determined during the process of tagging and measuring the lamb in the early post-partum period. These included estimating the approach distance which was the minimum distance before the ewe left the lamb on approach by a human and the maximum distance from the lamb the ewe retired to during lamb tagging and measurement. The actual time to return to the lamb after departure of the human was the final measure. None of these measures were affected by the temperament of the ewe and lamb. These results contrast with the results of Murphy (1999) which indicated that the time to return to lamb was greater in the nervous ewes than in the calm ewes.

Ewe behaviour was not significantly influenced by either temperament or litter size (Table 6). For *Cathair Donal*, flock of origin was found to be significant for approach distance. The distance before the ewes left their lambs on approach was higher for the *Grindon* ewes compared to ewes from the other two flocks of origin. There were non-significant trends for these ewes to be further away from the lamb and have a longer latency to return to the lamb. These results suggest that the ewes from *Grindon* were more fearful of humans.

Table 6 Effect of temperament, litter size and flock of origin (*Cathair Donal* only) on ewe behaviour during lamb tagging

	Approach distance (m)*	Max. distance from lamb (m)*	Time to return (s)*
Washpool			
Temperament	ns	ns	ns
Good	4.1	16.6	16.6
Poor	4.7	24.3	24.3
Litter size	ns	ns	ns
1	4.9	16.6	16.4
2	4.0	24.3	24.8
Cathair Donal			
Temperament	ns	ns	ns
Good	7.69	23.1	56.3
Poor	7.61	21.5	37.3
Flock of origin	P<0.01	ns	ns
Billandri	5.75[a]	18.0	45.1
Merinotech	6.55[a]	21.1	35.9
Grindon	11.94[b]	29.4	58.6
Litter size	ns	ns	ns
1	7.46	19.1	40.4
2	7.77	26.0	52.5

* Data had to be log-transformed prior to analysis and back-transformed means are presented.

Lamb temperament
The results for agitation score during the isolation box test (IBT) at weaning are presented in Table 7.

Ewe temperament influenced IBT agitation score of their progeny. This effect was significant in the *Washpool* weaners and only just failed to achieve significance (P=0.06) in the *Cathair Donal* weaners. This aligns with the results of Blache and Ferguson (2006) where IBT agitation score was found to be a moderately heritable trait. IBT agitation score was not affected by litter size, sex or flock of origin. Although the effect of sex was not found to be significant, the general trend that females have higher agitation scores is consistent with the Stage 1 results (refer Appendix 9.1) and those from a previous study where approximately 17,000 weaner sheep (wool, terminal sire and maternal flocks) were phenotyped for temperament using the IBT (Blache and Ferguson 2006).

Table 7 Effect of ewe temperament, litter size and flock of origin (*Cathair Donal* only) on weaner IBT agitation score.

	IBT agitation score*
Washpool	
Temperament	$P<0.05$
Good	30.6
Poor	46.1
Litter size	ns
1	34.5
2	40.4
Sex	ns
M	33.4
F	41.7
Cathair Donal#	
Temperament	$P=0.06$
Good	45.2
Poor	54.6
Flock of origin	ns
Billandri	47.5
Merinotech	51.4
Grindon	50.4
Litter size	ns
1	49.4
2	49.4
Sex	ns
M	47.0
F	52.5

* Data had to be log-transformed prior to analysis and back-transformed means are presented.
# Includes progeny from the early pregnancy group.

Conclusions – Stages 1 and 2

Temperament as determined by agitation score in the IBT is a heritable trait. However, the results from the Stage 1 and 2 components of this sub-project do not support the hypothesis that temperament affects lamb survival. Furthermore, post-partum ewe behaviour during lamb tagging (Stage 2)was not affected by temperament. Whilst it is reasonable to suggest that far greater selection pressure for temperament over several generations would be required before an effect on maternal behaviour and/or lamb survival might be observed, the recent results from the UWA selection line flock (Section 4.2.4) suggest that such associations may still be inconsistent.

Temperament is the behavioural expression of the intrinsic fearfulness of the animal when exposed to human contact or novel situations such as isolation. The evidence presented here indicates that the trait does not have a significant influence over maternal behaviour during the establishment of the ewe-lamb bond and therefore, lamb survival.

4.2 Sub-project B – Influence of genetic and environmental factors on maternal behaviour and physiology

The research from sub-project B has or is in the process of being published. These papers and draft manuscripts are attached as Appendices 9.2, 9.3, 9.4, 9.5 and 9.6. For the purposes of this final report only a summary of key outcomes are provided in the following as the complete results and discussion can be sourced from the papers and manuscripts.

4.2.1 B.1 Interaction between genotype and maternal behaviours on expression of temperament

A complete discussion of the results is provided in the journal manuscript attached as Appendix 9.2 - Genotype rather than non-genetic behavioural transmission determines the temperament of Merino lambs (accepted in *Animal Welfare*).

In summary, there was a genotype effect but no maternal or fostering effect on the lamb temperament at one-week of age. This may be because the maternal behaviour of the foster ewes did not differ considerably between the calm and nervous mothers during adoption or within the first week post partum. Similarly at weaning, only a genotype effect was found on the locomotor and agitation score.

It was concluded that temperament in Merino sheep is mainly determined by the genetic transmission of the trait across generations rather than behaviours learned from the mother.

4.2.2 B.2 Effect of social isolation and temperament on maternal behaviour

B2a
This research has now been published (Appendix 9.3 - Bickell et al (2009) Temperament does not affect the overall establishment of mutual preference between the mother and her young in sheep measured in a choice test. *Developmental Psychobiology* 51: 428-438*).*

In summary, ewes and lambs from both genotypes showed a similar preference for their familiar kin. In contrast, differences in temperament were detectable at one week and 16 weeks of age. Nervous lambs showed higher vocal and locomotor activity than calm lambs. It was concluded that temperament alone does not affect significantly the establishment of mutual preference between the ewe and her newborn lamb even though there is evidence suggesting that certain dimensions of temperament are associated with infantile and maternal behaviour such as proneness-to-distress during social separations (e.g. the open field test, the box test or the arena test) or alertness (two-choice test). Although these temperament dimensions did not predict the overall establishment of mutual preference under optimal lambing conditions (indoors) they might affect the strength of the ewe-lamb bond when it is established under more challenging conditions (outdoors).

B2b
In summary, selection for a calm or nervous temperament does not profoundly affect the intrinsic ability of mothers to display adequate maternal behaviour. The hormonal

differences between the two temperament lines were generally small and their possible influence on the display of maternal behaviour could not be demonstrated in the present study. The complete results are presented in draft journal manuscript attached as Appendix 9.4.

B2c
This research was published by Hart et al (2006) (see Appendix 9.5 Colostrum quality of ewes of calm temperament is not responsible for low mortality. *Aust. J. Exper. Agric.* **46**:827-829).

Calm temperament was found to have an influence on the viscosity of colostrum 6 h after birth. High variability in all aspects of colostrum production was found in the study and this may be a limiting factor in lamb survival under certain circumstances. It was concluded however, that there is little evidence to suggest a strong association with ewe temperament and colostrum production and therefore, this could, in part, explain lower lamb mortality that is reported to occur among calm ewes.

4.2.3 B.3: Effect of temperament on ewe-lamb discrimination in the face of novel stressors

At 6 h after parturition, the time to first reach a lamb, amount of time next to or looking at each lamb, number of vocalisations, number of visits were not different between calm and nervous mothers. Both calm and nervous ewes spent more time near their own lamb than near the alien lamb (Calm p = 0.01; Nervous p = 0.03). At 18 h after parturition, calm lambs spent more time to reach their own mother than the nervous. The number of vocalisations and the number of visits to their mother were not different between calm and nervous lambs. The number of high pitched bleats (distress calls) were not different between the lambs of the two lines. We conclude that the selection for a nervous temperament does not seem to impair the capacity of ewes and lambs to display an early preference for each other. In fact, having a nervous temperament may prove to be an advantage in some challenging situations since it seems to increase the motivation of nervous lambs to reunite with their mother (see Appendix 9.6 for draft manuscript).

4.2.4 B.4 Effect of temperament on maternal behaviour expressed during parturition and bonding

Parturition
The proportion of ewes giving birth during the day or night during 2008 did not differ between temperament lines ($\chi^2 = 0.00$; p = 0.999) or experience ($\chi^2 = 0.48$; p = 0.49). Forty-one percent of both calm and nervous ewes gave birth during the day, while 59% of both calm and nervous ewes gave birth at night. Forty-seven percent of maidens and 40 % of experienced ewes gave birth during the day, while 53% of maidens and 50% of experienced ewes gave birth at night. The length of labour also did not differ between temperament lines (p = 0.708, Table 8) or between maiden or experienced ewes (p = 0.865, Table 8).

Mortality rate
There was a tendency (p = 0.087) for a higher rate of mortality in nervous lambs (18%) compared with calm lambs (11%) in 2008. However, there was no effect of temperament on the number of lambs that died between birth to weaning (16 weeks) in 2007 (Calm = 18.2%, Nervous 17.7%; p = 0.906).

There was no effect of previous lambing experience of the ewe on the mortality rate of the lambs. Twenty-two percent of lambs born to maiden mothers and 16% born to experienced mothers died in 2007 ($p = 0.221$), while 19% of lambs born to maiden mothers and 17% born to experienced mothers died in 2008 ($p = 0.738$). Regardless of temperament or experience, lamb mortality did not differ ($p = 0.303$) between 2007 (18%) and 2008 (15%).

Comparison of ewe behaviour between temperament lines
The behaviour of the ewe did not differ markedly between temperament lines during the two hours postpartum (Table 8). Calm ewes licked their lambs more than the nervous ewes ($p < 0.001$) while the time ewes spent licking their lambs decreased over time for all ewes ($p < 0.001$). There was a tendency ($p = 0.076$) for calm ewes to take longer than nervous ewes to accept their lamb at the udder. However calm and nervous ewes did not differ in the time taken to accept their lamb at the udder relative to the time taken for the lamb to start searching for the udder ($p = 0.467$). There was also a tendency ($p = 0.066$) for nervous experienced ewes to look around more than calm experienced ewes while calm maiden ewes looked around more than nervous maiden ewes. This behaviour of the calm mother could mean that they are more vigilant than the nervous mother, however, this interpretation needs to be further demonstrated. Calm and nervous ewes did not differ in the time they spent grazing ($p = 0.907$), in the number of times a ewe stops her lamb from suckling relative to the time the lamb spends searching for the udder ($p = 0.549$), the number of times a ewe backs away or circles away from her lamb relative to the time the lamb spends searching for the udder ($p = 0.911$; $P = 0.834$).

The time in which ewes moved >3m away from the birth site tended to differ between temperament lines ($p = 0.087$). Calm ewes tended to spend longer on the birth site than nervous ewes (Calm = 114.9 ± 15.5 min, Nervous = 80.8 ± 10.9 min).

Comparison of ewe behaviour between maiden and experienced ewes
Previous lambing experience influenced the behaviour of the ewes during the two hours postpartum. Experienced ewes spent more time grazing than maiden ewes ($p = 0.039$), while the time spent grazing increased over time for all ewes ($p < 0.001$). Maiden ewes took longer than experienced ewes to accept their lambs at the udder ($p = 0.026$). However maiden and experienced ewes did not differ in the time taken to accept their lamb at the udder relative to the time taken for the lamb to start searching for the udder ($p = 0.305$). Maiden ewes also had a higher ratio of backing or circling away from their lamb relative to the time the lamb spent searching for the udder ($p = 0.026$; $p = 0.011$). However, there were no differences between maiden and experienced ewes in the time spent licking their lamb ($p = 0.357$) or in the number of times a ewe stops her lamb from sucking relative to the time the lamb spends searching for the udder ($p = 0.933$).

The time in which ewes moved >3m away from the birth site did not differ between experience (Maiden = 103.1 ± 1.7 min, Experienced = 93.5 ± 11.9 min; $p = 0.624$).

Comparison of lamb behaviour between temperament lines
Calm lambs took longer to stand up ($p < 0.001$) than nervous lambs and also tend ($p = 0.07$) to take longer in attempting to stand (Table 8). Calm lambs also took longer to start searching for the udder ($p = 0.048$) than nervous lambs. However calm and nervous lambs did not differ in the latency to start searching for the udder relative to the latency to stand ($p = 0.226$). Nervous lambs spent more time over the 2 hour

observation period searching for the udder (p = 0.07). There was also no difference between temperament lines in the time lambs spent suckling at the udder relative to the time spent searching for the udder (p = 0.568). Calm and nervous lambs also do not differ in the time they spent lying (p = 0.115) or the time they spent being inactive while standing (p = 0.24).

Comparison of lamb behaviour between lambs born from maiden and experienced ewes

The previous lambing experience of the ewe did not affect the behaviour of the lamb (Table 8). Lambs born from maiden or experienced ewes did not differ in the time taken to attempt to stand (p = 0.517), stand (p = 0.595), start searching for the udder (p = 0.377), time spent searching for the udder (p = 0.547), time spent suckling at the udder relative to the time spent searching for the udder (p = 0.146), time spent lying (p = 0.317) or being inactive while standing (p = 0.24).

Table 8 Ewe and lamb behaviour, mean ± s.e.m (n), in outdoor lambing conditions from birth - 2 hours postpartum.

	Calm	Nervous	Maiden	Experienced
Ewe Behaviour				
Length of labour (min)	65.4 ± 9.6 (37)	70 ± 11.5 (39)	70.1 ± 13.8 (27)	66.5 ± 8.9 (49)
Time licking lamb (min)	5 ± 0.2[a] (55)	3.8 ± 0.2[b] (51)	4.5 ± 0.3 (35)	4.3 ± 0.2 (72)
Time grazing (min)	1.9 ± 0.3 (27)	2.3 ± 0.3 (21)	1.4 ± 0.4[c] (16)	2.4 ± 0.3[d] (32)
Time looking up (min)	2.9 ± 0.3 (27)	3.5 ± 0.3 (21)	3.3 ± 0.5 (16)	3.1 ± 0.3 (32)
Lat. to accept lamb at udder (min)	47.4 ± 4.0 (56)	36.2 ± 3.7 (44)	50.4 ± 4.2 (29)	39.2 ± 3.4 (71)
Lat. to accept lamb at udder / Lat. to stand	2.2 ± 0.2 (54)	3.2 ± 0.4 (41)	3.5 ± 0.5 (28)	2.3 ± 0.3 (50)
Stops lamb suckling / time lamb searches for udder	1.4 ± 0.2 (26)	2.0 ± 0.4 (21)	1.6 ± 0.3 (16)	1.7 ± 0.3 (31)
Backs away from lamb / time lamb searches for udder	0.7 ± 0.2 (26)	1.1 ± 0.5 (21)	1.5 ± 0.5[c] (16)	0.6 ± 0.3[d] (31)
Circles away from lamb / time lamb searches for udder	4.2 ± 1.1 (26)	2.8 ± 1.3 (21)	6.4 ± 1.9[c] (16)	2.1 ± 0.7[d] (31)
Lamb behaviour				
Lat. to attempt to stand (min)	12.8 ± 1.6 (64)	9.2 ± 1.2 (49)	12.3 ± 2.2 (37)	10.7 ± 1.1 (76)
Lat. to stand (min)	23.9 ± 1.9[a] (65)	15 ± 1.6[b] (53)	20.8 ± 2.3 (36)	19.5 ± 1.6 (82)
Lat. to search for udder (min)	29.5 ± 3.0[a] (54)	20.3 ± 1.9[b] (48)	27.9 ± 3.3 (30)	24 ± 2.2 (72)
Lat. to search for the udder / Lat. to stand	1.3 ± 0.08 (52)	1.4 ± 0.1 (47)	1.5 ± 0.2 (30)	1.3 ± 0.1 (57)
Time searching for udder (min)	3.4 ± 0.3[a] (26)	4.3 ± 0.4[b] (21)	4 ± 0.3 (16)	3.7 ± 0.3 (31)
Time suckling at udder (min)	0.5 ± 0.1 (26)	0.6 ± 0.1 (21)	0.5 ± 0.1 (16)	0.6 ± 0.07 (37)
Time suckling / time searching for udder	0.2 ± 0.04 (26)	0.1 ± 0.042 (21)	0.1 ± 0.04 (16)	0.2 ± 0.03 (37)
Time lying (min)	4.1 ± 0.3 (26)	3.6 ± 0.4 (21)	4.3 ± 0.4 (16)	3.7 ± 0.3 (31)
Time standing inactive (min)	2.5 ± 0.3 (26)	2 ± 0.3 (21)	1.8 ± 0.3 (16)	2.5 ± 0.3 (31)

[a] and [b]: comparisons to test the effect of temperament; [c] and [d]: comparisons to test the effect experience; row-means with different superscripts are significantly different (P < 0.05).

Discussion

Under natural, undisturbed lambing conditions calm and nervous ewes show minor behavioural differences. Calm ewes lick their lambs more and tend to spend longer on the birth site compared with nervous ewes. Our results do follow that same trend found by Murphy (1999), and the tendencies may have become significant had we used larger sample sizes. Despite the minor behavioural differences between calm and nervous ewes, calm lambs still tended to have higher survival rates in 2008 than nervous lambs. The fact that higher rates of survival occurred during one year and not the other suggests there were seasonality factors impacting on the survival of the lamb during 2008, but not during 2007. In addition, there seems to be an effect of temperament on the behaviour of the lamb. Nervous lambs are more active but seem to be less efficient at suckling than calm lambs. Overall we did not see any major effect on ewe behaviour and no effect on lamb survival. The selection for nervous lambs in theory should give them an advantage as they stand earlier than calm lamb. On the other hand, they take more time to find the udder so they have a greater chance to miss some crucial first feed.

It is concluded that the higher rate of lamb survival in the calm lambs might be due to complex interactions between temperament, ewe and lamb behaviour, seasonality and other factors affecting lamb survival rather than just a of few specific behaviours.

5 Success in Achieving Objectives

1. To determine whether selection for temperament will improve reproductive efficiency and neonatal lamb survival in commercial wool and meat sheep flocks.

The research was successfully completed however the results did not show that either screening for temperament (Stage 1) or selection for temperament over one generation (Stage 2) led to improved lamb survival in wool and meat sheep (Stage 1 only) flocks.

2. To investigate the interaction between genetic and environmental factors on the establishment of the ewe-lamb bond and subsequent lamb survival.

All research was completed and three journal papers have been published or accepted for publication and a further two journal manuscripts have been submitted.

The key findings were:

- Expression of temperament is genetically controlled rather than being a behaviour learned from early interactions with the ewe.
- Several behavioural factors between the ewe and lamb previously thought to be relevant to the differences in lamb survival between the temperament selection lines were not found to be important.
- Nervous ewes produced thicker colostrum and their lambs took more time to find the udder. These factors may result in reduced or retarded supply of nutrients to the lamb and consequently, could have a bearing on its survival.

3. To develop improved on-farm management strategies (based on the outcomes of objectives 1 and 2) that focus on managing the pregnant ewe and increasing lamb survival.

There was no compelling evidence to support the recommendation of selection for temperament on-farm as a means to improve lamb survival. This is not to say that temperament is completely irrelevant, rather its influence on the expression of maternal behaviour was not strong.

Consequently, no new recommendations can be made with regard to the management of the pregnant ewe other than those that have been well-documented and proven (Geenty 1997). To that end, it is recommended that there is still an urgent need to increase the on-farm adoption of simple strategies such as ultrasound pregnancy scanning, improved nutritional management of the gestational ewe and predicting and mitigating the impact of cold stress events during lambing.

4. To train 2 PhD students in the field of farm animal welfare.

This objective is partially completed. Miss Samantha Bickell is in the final stage of completing her PhD thesis. She has published three papers and submitted a further two journal manuscripts from her research. Samantha was awarded an extension of her scholarship by UWA and she will submit her thesis by July 2009.

Mr Ken Hart is trying to complete his PhD in a part-time capacity having returned to full-time work with DAFWA in February 2008. Ken has published one paper and has submitted another paper to AAABG. It is anticipated he will submit his PhD thesis by February 2010.

6 Impact on Meat and Livestock Industry – now & in five years time

There is a need to improve lamb survival in sheep flocks particularly wool flocks produced under more extensive management systems. The high lamb mortality (>20%) that can and does occur in Australian sheep flocks results in substantial production and economic losses but perhaps more importantly it remains a significant animal welfare issue.

In terms of improving lamb survival, selection for improved temperament is not likely to achieve significant improvements. Rather, greater effort should be invested in increasing the adoption of well proven on-farm practices such as ultrasound pregnancy scanning, improved nutritional management of the gestational ewe and predicting and mitigating the impact of cold stress events during lambing. An increase in adoption of these simple yet effective strategies is likely to result in a substantial improvement in lamb survival in Australian flocks.

7 Conclusions and Recommendations

Based on the combined results from the two sub-projects, it is concluded that selection for temperament is not likely to significantly alter maternal behaviour during and immediately subsequent to parturition. Therefore, we conclude that as an on-farm strategy, selection for temperament will not result in significant improvements in lamb survival. Small gains in colostrum quality and neonatal suckling behaviour/vigour, which are important to lamb survival, may arise but only in those flocks where there has been significant selection intensity for temperament over several generations.

It is our recommendation that whilst there is scope for genetic improvement in reproductive traits like lamb survival, greater gains will be achieved through increased adoption of existing knowledge and strategies centred on improved nutrition and management of the gestational and post-partum ewe. There are three obvious strategies that if applied more widely would bring about immediate improvements in lamb survival. These include:

(i) Pregnancy scanning – mid-pregnancy scanning for pregnancy status and litter size not only provides a more accurate measure of the expected or potential lamb numbers but it enables multiple bearing ewes to be identified and therefore managed separately given their high nutritional demands.

(ii) Improved nutrition of the gestational ewe.
The evidence demonstrating the impact of improved nutrition and maintaining ewes at a body conditions score of ≥ 2.5 on lamb survival is well documented.

(iii) Predicting and mitigating the impact of cold stress at lambing.
For example, losses due to hypothermia (starvation/exposure) could be reduced through better weather forecasting systems and increasing available shelter in lambing paddocks.

It is recognised that efforts to increase the adoption of these practices is currently underway within the CRC for Sheep Industry Innovation.

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9 Appendices

9.1 Appendix A1 Milestone 6.1 Report - Report on the fixed effects analysis of the effect of temperament on reproductive losses on farm and summary of all data collected, results and findings to date.

MLA project code:	**AHW.085**
MLA project title:	**Improving lamb survival by selection for temperament**
Project leader:	**Dominique Blache/Drewe Ferguson**
MLA project manager/coordinator:	**Danielle Marotti**
Milestone number:	**6.1**

Abstract

In Part A of this project, the aim was to examine the associations between temperament, maternal behaviour and lamb survival. Ewes (n = 5791) from seven commercial flocks (4 Merino and 3 Terminal sire) were screened for temperament using the IBT and joined by AI to rams which had high or low EBVs for temperament. Within each flock, a subset of unscreened ewes was randomly selected as the control group. An associative mating program was used to generate three temperament groups Poor, Good and Control. Industry sires were used to join the control ewes and they also provided linkage across the 4 Merino and 3 Terminal sire flocks. The reproductive losses were then quantified. The pregnancy rate (No. of foetuses/pregnant ewe %) was moderately high ranging from 126.9 – 160.1% for Merinos and 146.3 -167.4% for Terminal sire flocks. This was attributed to higher multiple pregnancies Lamb survival was significantly influenced by flock ($P<0.001$) and litter size ($P<0.001$) but not temperament group. Lamb survival (No. of lambs at marking/No. of expected lambs %) ranged from 46.2 – 72.9 % and 52.2 – 71.7% for the Merino and Terminal sire flocks, respectively. The results for each flock were: Billandri (wool WA) 49.7%, Merintech (wool WA) 72.9%, Grindon (wool WA) 46.2%, Washpool (wool SA) 70.6, Genstock (teminal WA) 63.5%, Gnaring Park (terminal WA) 52.2% and Eagleridge (terminal VIC) 71.7%. Overall lamb survival was quite low in most of the WA flocks (Merino and Terminal sire) and this was directly linked to the poor pasture conditions during gestation despite supplementary feeding in some cases. There was a commensurate decrease in lamb survival with an increase in litter size. The IBT agitation score of the progeny was significantly affected by flock ($P<0.001$ Merino and Terminal sire), temperament group ($P<0.05$ Merino; $P=0.06$ Terminal sire) and sex ($P<0.01$ Merino; $P<0.001$ Terminal sire). A significant interaction between flock x temperament group ($P<0.05$) was also observed for the Merino flocks. Importantly, genetic divergence in temperament was evident in the Merino F1 progeny which was important as these ewes will be joined in 2008 to further test the hypothesis that temperament affects maternal behaviour and this in turn, will have a significant effect on lamb survival.

Project objectives

1. To determine whether selection for temperament will improve reproductive efficiency and neonatal lamb survival in commercial wool and meat sheep flocks.

2. To investigate the interaction between genetic and environmental factors on the establishment of the ewe-lamb bond and subsequent lamb survival

3. To develop improved on-farm management strategies (based on the outcomes of objectives 1 and 2) that focus on managing the pregnant ewe and increasing lamb survival

4. To train 2 PhD students in the field of farm animal welfare

Success in achieving milestone

Milestone 6.1: Report on the fixed effects analysis of the effect of temperament on reproductive losses on farm and summary of all data collected, results and findings to date.

Introduction

The establishment of a strong ewe-lamb bond during the first hours after birth is central to lamb survival. There are many factors which are inherent in both the dam and lamb that are crucial to this early post-partum process (for review see Nowak *et al* 2004). Moreover, it is well recognised that the individual dam and lamb factors are synergistic.

A key factor here is the expression of maternal behaviour which is regulated by several neuroendocrinal modulators. This expression can be negatively affected by the innate fearfulness or temperament of the ewe. For example, immediately prior to parturition the ewe separates from the flock. This of course is incongruent with her natural flocking behaviour. Her capacity to cope with this internal conflict between the stress of parturition and isolation from the flock will have a huge bearing on the expression of maternal behaviour and ultimately lamb survival. This is perhaps best illustrated by the results of Murphy (1999) who showed a twofold difference in lamb mortality between selective breeding lines for low and high reactivity to isolation and human contact (Murphy 1999). A key outcome of the research using this unique resource was that the less reactive ewes were clearly better mothers in that they spent more time with their lambs, had shorter flight distances when disturbed by humans and returned to their lambs faster than more reactive ewes (Murphy, et al. 1994; Murphy 1999).

Selection for temperament may therefore have a positive effect in terms of the expression of maternal behaviour and therefore lamb survival. The primary aim of Part A of the project was to test this hypothesis in commercial wool and meat flocks.

Material and methods

Flock details and experimental procedures
The details of the seven flocks and management of the ewes were detailed in a previous milestone reports (MR2 and 3)(see Annex 1). Further information on the nutrition and management of the ewes during gestation and lambing is summarised in an Annex 2.

Briefly, the seven flocks (5 WA, 1 SA and 1 Vic) comprised four wool and three prime lamb flocks. Approximately 800-1000 ewes/flock were used in the experiment (Total = 6200 ewes). Of these, a group of approximately 200-300 ewes were randomly drafted off as the control group. The remainder were tested for temperament using the isolation box test (IBT) and the 200-300 ewes with the lowest and highest IBT scores were selected as the good and poor temperament groups, respectively. Blood samples (6ml EDTA vacutainers) were collected from these ewes by jugular venipuncture.

A total of 5791 ewes were subsequently artificially inseminated to selected sires. An associative joining program was used where ewes from the good temperament group were joined to sires with low EBVs for IBT agitation score (ie. good temperament). Similarly, sires with high EBVs for the trait (ie. poor temperament) were used in the poor temperament ewe group. The sire EBVs for IBT agitation score is shown in Table 1.

Ewes from the control group were artificially inseminated (AI) with semen from industry sires (3 Merino and 2 Poll Dorset) which had no progeny data on temperament. These sires also served as link sires across the flocks within each production system (wool and prime lamb).

Table 1 Sire EBVs for IBT agitation score within breed.

Breed	Sire ID	IBT Agitation Score EBV	Temperament Group
Merino	5003831999995873	-30.51	Good
	5030542001010228	-38.41	Good
	5030702002020150	-30.02	Good
	6012502001107439	-35.65	Good
	6012502001107448	-42.90	Good
	501587199999J665	-48.60	Good
	5047671999OR0382	-28.03	Good
	5030541997970186	63.72	Poor
	5049011999990075	18.87	Poor
	6005711999990354	38.41	Poor
	6009992000000189	32.91	Poor
	6010822000001217	38.46	Poor
	6012502001107351	51.82	Poor
	5047672001VI0172	38.01	Poor
Poll Dorset	1601852003030236	-20.57	Good
	1622882002020099	-25.33	Good
	1640732002020433	-32.98	Good
	1618922002020555	26.81	Poor
	1623682001010351	13.63	Poor
	1640732002020284	34.57	Poor
White Suffolk	2300262001012020	-30.24	Good
	2300992003030135	-34.34	Good
	2301132002020124	20.72	Poor
	2301222001011131	30.82	Poor

The ewes were pregnancy scanned approximately 60 days after AI. After drafting off the non-pregnant ewes or ewes assessed as pregnant to back-up rams, the remaining ewes were separated into two groups based on scanned litter size (single and multiple bearing ewes). These two groups, which included all three temperament treatments (Good, Poor and Control) were managed separately.

During gestation through to lambing, basic information on the pasture and climate conditions was captured. During gestation, it was necessary to provide supplements to the ewes on all seven collaborating farms. The type and amount of supplement offered was recorded.

During the lambing phase (Day 145 – 155 after AI), human contact with the ewes was kept to a minimum. The lambing paddocks were monitored daily and tissue samples from lambs found dead were collected for DNA extraction.

At marking, the lambs were ear tagged, weighed and a sample of blood (6ml EDTA vacutainer by jugular venipuncture) was collected for parentage assignment. The lambs were returned to the ewes until they were weaned. Within two weeks after weaning, the IBT agitation score and liveweight of the lambs was recorded.

The live weight of the ewes was recorded at three time points, during temperament screening, scanning and at lamb marking.

Parentage assignment
DNA was extracted using QIAGEN DNeasy Blood and Tissue kits according to the procedures of the manufacturer.

A sample of blood from each ewe and lamb was transferred onto cards from the Catapult® test kits. These cards plus semen from all 29 rams and extracted DNA (dead lambs) were sent to Catapult Genetics for parentage assignment of all lambs.

Statistical analysis
After collating the data and identifying records where the parentage of the lambs could not be assigned, the database was created based on the scanned records. Each lamb identified at scanning was given a unique identification. A further 146 records were added to the database which represented additional lambs that were not detected at scanning but were identified through DNA parentage assignment. These lambs were typically from ewes where the litter size had been underestimated by the scanner.

Within the database, lamb survival was defined as a binomial trait where each record was assigned either 0 (dead or missing) or 1 (alive at marking).

For the analysis of lamb survival, the mixed model procedure in SAS (SAS 1999) was used where flock, temperament group and litter size were included as fixed effects and sire and dam were included as random effects. Separate analyses were performed for the wool and meat flocks. The model used to analyse the trait IBT agitation score in the live progeny included the fixed effects of flock, temperament group, sex and the random terms sire and dam. The covariate weaner liveweight was also fitted in this model.

Results and Discussion

(i) Reproductive losses

The summary of results for each flock is presented in Table 2. There are a number of key points to highlight with respect to these results. There were a number of live (n = 447) and dead (n = 142) lambs which could not be correctly assigned to sire and dam. This was mainly due to a failure to accurately assign the dam rather than the sire in the majority of instances. Secondly, there were 813 scanned foetuses that were not recovered. A proportion of these would have been lost during gestation (day 60 – 150) although losses during this period are generally low <2% (Kelly 1983, Smith et al 1988). However, we believe that the majority of these would have gone to term and probably died during or within 3-5 days after parturition. During lambing, human contact was kept to a minimum to reduce any external stimuli on the ewes during the establishment of the maternal bond. Lambing rounds were only performed once daily and therefore it is most likely these lambs were not recovered due to predation. Thirdly, with respect to the reproductive components the percentage of expected number of lambs/ewe pregnant was relatively high (Merino 124.4 – 159.4%; Prime lamb 145.9 -167.1%). The increased prolificacy (ie. twins and triplets) observed here is most likely due to the effect of the Folligon® treatment given to the ewes in preparation for artificial insemination. As a contrast, Kleeman and Walker (2005) reported that the percentage of expected lambs/ewe pregnant in Merino flocks where natural mating was used was 126.9%. The percentage of expected lambs that were alive at marking was quite variable between flocks. The results for two of the Merino flocks (Merinotech and Washpool) were comparable to other published estimates (64-82% Kilgour (1992), 72.5% Kleeman and Walker (2005)). However, for the remaining Merino flocks (Bilandri and Grindon) the reproductive losses were considerably higher and this can be attributed to the poor pasture conditions encountered during the first 6 months of 2006 in Western Australia. This is further emphasised by the high number of ewes that died during lambing in both these flocks. Kleeman and Walker (2005) reported an average ewe mortiality rate of 1.4% but it ranges from 0.0 – 15.6%. The poor pasture conditions were also undoubtedly a key factor for the lower lambing percentages on the two prime lamb flocks in WA (Genstock and Gnaring Park). It is worth noting that supplementation was provided during gestation in all seven flocks (refer Annex 2) but it would appear that this was insufficient to maintain ewe condition in some flocks. The level of supplementation was at the discretion of the collaborating owner or farm manager.

To further reinforce the effect of the pasture conditions, the mean loss in liveweight in the ewes/flock between temperament screening and lamb marking expressed as a percentage of the first liveweight accounted for 21% of the variance in number of live lambs at marking/number of expected lambs percentage (Figure 1). The actual change in liveweight between temperament screening and lamb marking is shown in Table 2.

In a study by Kelly (1992) which examined lamb mortality in commercial Merino flocks in WA, he identified a stronger correlation between liveweight mid-pregnancy and mortality in both single (R^2=0.41) and twin (R^2 = 0.57) born lambs. Collectively, these results reinforce the paramount importance of maintaining ewe body condition during pregnancy to maximise lamb survival.

When quantifying lamb survival, the number of live lambs expressed as a percentage of expected lambs (based on scanning) is probably the most definitive and accurate benchmark. This point was also made by Kilgour (1992). Historically, within the sheep industries the ratio of number of live lambs at marking/number of ewes joined or number of live lambs at marking/number ewes at marking has been favoured but both these measures underestimate reproductive wastage.

Table 2 Mean ± se liveweights of the ewes in each flock at temperament testing[1] and lamb marking[2]

Flock	Mean ± se ewe weight Temperament testing	Mean ± se ewe weight Lamb marking
Merino flocks		
Bilandri	60.3 ± 0.2	53.7 ± 0.2
Merinotech	62.6 ± 0.2	50.2 ± 0.2
Grindon	50.7 ± 0.2	44.3 ± 0.3
Washpool	50.9 ± 0.3	49.6 ± 0.3
Terminal sire flocks		
Genstock	65.5 ± 0.2	61.3 ± 0.2
Gnaring Park	55.0 ± 0.3	41.4 ± 0.3
Eagleridge	54.6 ± 0.3	56.3 ± 0.3

[1] Refer Annex 1 for dates for each flock
[2] Refer Table for dates for each flock

Although not shown in Table 3 the losses in lambs between weaning and marking were 13, 58, 66 and 7 for the four Merino flocks and 53, 29 and 16 for the Terminal sire flocks.

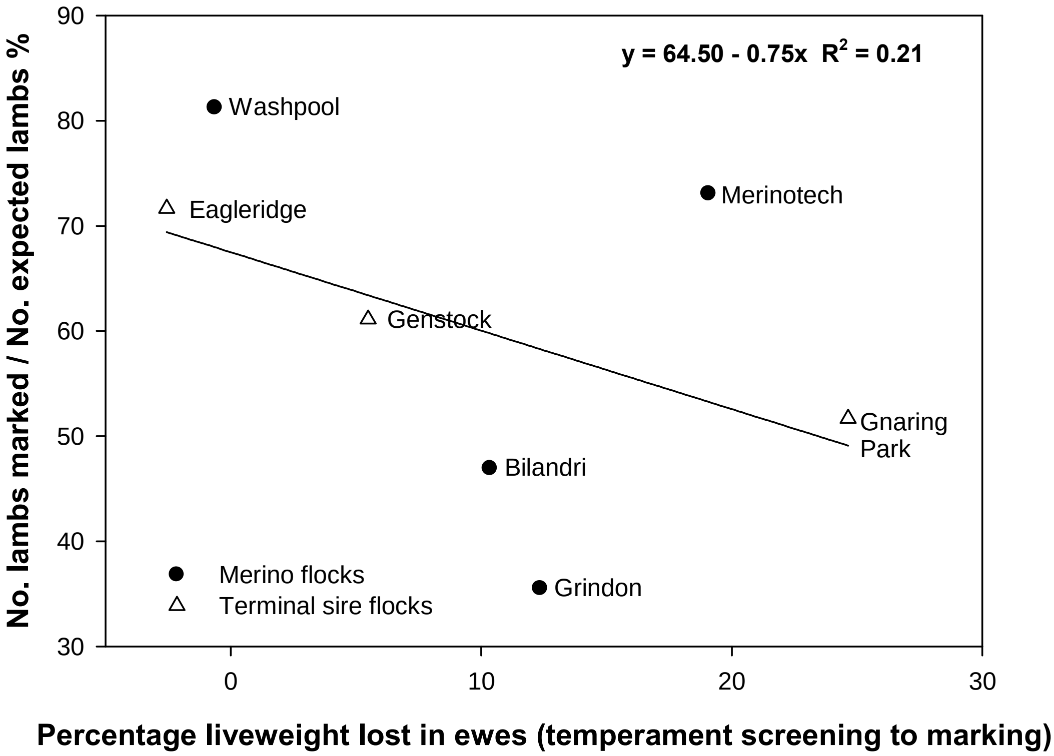

Figure 1 Association between percentage liveweight lost in ewes between temperament screening and marking and lamb survival

Table 3 Summary of the reproductive and lambing results for each flock

	1	2	3	4	5	6	7
Owner	**Sandilands**	**Robertson**	**Ritson**	**Hocking-Edwards**	**Hegarton**	**Watts**	**Sargeant**
Flock	**Billandri**	**Merinotech**	**Grindon**	**Washpool**	**Genstock**	**Gnaring Park**	**Eagleridge**
Production System	**Wool**	**Wool**	**Wool**	**Wool**	**Prime lamb**	**Prime lamb**	**Prime lamb**
AI Date	*13-15 Feb 06*	*1-3 Feb 06*	*20-22 Feb 06*	*19-20 Jan 06*	*23-24 Jan 06*	*4-6 Jan 06*	*31 Jan - 2 Feb 06*
Lambing starts (day 147 after first day of AI)	*31-May-06*	*19-Jun-06*	*28-Jun-06*	*10-Jul-06*	*17-Jul-06*	*27-Jun-06*	*15-Jun-06*
Marking dates	*05-Jul-06*	*07-Jul-06*	*18-Jul-06*	*14-Aug-06*	*04-Sep-06*	31-Jul-06	*16-Aug-06*
No. of ewes joined	705	893	856	870	751	876	834
No. of ewes pregnant	512	622	568	577	621	551	641
% Ewes pregnant	**72.6**	**69.7**	**66.4**	**66.3**	**82.7**	**62.9**	**76.9**
No. of foetuses scanned	816	911	793	718	1038	804	967
% foetuses/ewe joined	**115.7**	**102.0**	**92.6**	**82.5**	**138.2**	**91.8**	**115.9**
% foetuses/ewe pregnant	**159.4**	**146.5**	**139.6**	**124.4**	**167.1**	**145.9**	**150.9**
No. additional lambs* (not identified at scanning)	41	8	30	13	21	8	25
Expected total lambs	**857**	**919**	**823**	**731**	**1059**	**812**	**992**
No. of lambs not recovered (based on scanning records)	131	75	198	43	108	190	68
% lambs not recovered	15.29	8.16	24.06	5.88	10.20	23.40	6.85
No. of live lambs at marking with **known** parentage	426	670	380	516	672	424	712
No. of dead lambs with **known** parentage	197	125	135	73	154	130	177
No. of live lambs at marking with **unknown** parenatge	72	40	68	87	100	47	33
No. of dead lambs with **unknown** parentage	31	9	42	12	25	21	2
	726						
% live lambs/expected lambs	**49.71**	**72.91**	**46.17**	**70.59**	**63.46**	**52.22**	**71.77**
% live lambs/ewe joined	**60.4**	**75.0**	**44.4**	**59.3**	**89.5**	**48.4**	**85.4**
% live lambs/ewe pregnant	**83.2**	**107.7**	**66.9**	**89.4**	**108.2**	**77.0**	**111.1**
No. of dead ewes at lambing	20	8	60	4	8	44	3
% dead ewes	**2.8**	**0.9**	**7.0**	**0.5**	**1.1**	**5.0**	**0.4**

(ii) Effect of flock, litter size and temperament group on lamb survival

The results from the mixed model analyses for the wool and terminal sire flocks are presented in Table 4. The results from two separate analyses are presented. The first analysis (P+U) included records where the pedigree was known plus the records pertaining to the unrecovered lambs (as was presented in the initial MR6). The reason for including the unrecovered lambs was that the sire and dam were known and it is reasonable to assume that the majority of these died in the immediate post-partum period. The second analysis (P) was conducted on only those records where the parentage was known. Clearly, the latter analysis overestimates lamb survival.

As expected based on the results in Table 1, there were significant differences ($P<0.001$) between flocks in both productions systems. The flock differences were much larger in the Merino flocks.

Litter size had a very pronounced effect on lamb survival in both production systems ($P<0.001$) where survival decreased with increased litter size. The negative association between litter size and lamb survival agrees with the general dogma from a large number of studies (eg. Alexander 1983, Kilgour 1992, Kelly 1992, Kleeman and Walker 2005).

The differences in lamb survival between the temperament groups approached significance (P+U - $P=0.06$; P - $P=0.06$) in the case of the Merino flocks and was not significant for the terminal sire flocks. However, the trend for lamb survival was contrary to expectation where it was slightly higher in the poor compared to the good temperament group. Overall, the results were disappointing as a small difference between the temperament groups was expected. The large environment differences between flocks, particularly those in WA, may have negated any genetic differences due to temperament. Although we cannot conclude this definitively, greater control of the nutritional management of the F1 ewes for the 2008 lambing will be exercised to minimise this effect. A second factor that may have contributed to the result is that ewes were screened for temperament as adults. This may have introduced some noise or error in the phenotypic assessment. The preferred age to measure temperament is in the immediate post-weaning period when the inherent fearfulness of the animal is least likely to be influenced by experiential factors (Blache and Ferguson 2006). At this young age, the experiential effects on the expression of the trait are likely to be small. At an older age, the past experiences of the animal will have a larger influence on behaviour. Therefore, the trait when measured at an older age will be more variable due to the increased environmental influence.

Our expectation however, is that the effect of temperament on lamb survival is more likely to be evident in the F1 female progeny as there will be an increased genetic divergence in the trait compared to the parent dams.

Table 4 Least square means for the effect of flock, litter size and temperament group on lamb survival

	Lamb Survival (P+U) Signif. and LS means ± se	**Lamb Survival (P)** Signif. and LS means ± se
Merino	n = 3201	n = 2517
Flock	*P<0.001*	*P<0.001*
Bilandri	$0.51^{a} \pm 0.02$	$0.67^{a} \pm 0.02$
Merinotech	$0.71^{c} \pm 0.02$	$0.79^{b} \pm 0.02$
Grindon	$0.44^{b} \pm 0.02$	$0.68^{a} \pm 0.02$
Washpool	$0.75^{c} \pm 0.02$	$0.78^{b} \pm 0.02$
Temperament group	*P = 0.07*	*P= 0.06.*
Good	0.58 ± 0.02	0.74 ± 0.02
Control	0.59 ± 0.02	0.70 ± 0.02
Poor	0.63 ± 0.02	0.75 ± 0.02
Litter size	*P<0.001*	*P<0.001*
1	$0.76^{a} \pm 0.01$	$0.92^{a} \pm 0.01$
2	$0.55^{b} \pm 0.01$	$0.72^{b} \pm 0.01$
≥3	$0.49^{b} \pm 0.03$	0.56 ± 0.03
Terminal Sire	n = 2829	n = 2257
Flock	*P<0.001*	*P<0.001*
Genstock	$0.63^{a} \pm 0.02$	$0.79^{a} \pm 0.02$
Gnaring Park	$0.48^{b} \pm 0.02$	$0.68^{b} \pm 0.02$
Eagleridge	$0.68^{c} \pm 0.02$	$0.76^{a} \pm 0.02$
Temperament group	*n.s.*	*n.s.*
Good	0.61 ± 0.02	0.77 ± 0.02
Control	0.57 ± 0.02	0.71 ± 0.02
Poor	0.60 ± 0.02	0.74 ± 0.02
Litter size	*P<0.001*	*P<0.001*
1	$0.79^{a} \pm 0.02$	$0.93^{a} \pm 0.02$
2	$0.58^{b} \pm 0.02$	$0.75^{b} \pm 0.01$
≥3	$0.41^{c} \pm 0.02$	$0..55^{c} \pm 0.03$

Least square means that do not share the same superscript are significantly different
P+U – records from lambs with known parentage and unrecovered lambs (based on scanning data)
P – records from lambs with known parentage

(iii) Effect of flock, sex and temperament group on temperament

The agitation score in the progeny was significantly influenced by all main effects in both the Merino and terminal sire flocks (Table 5). A significant interaction between flock x temperament group (P<0.01) was also observed for the Merino flocks. This interaction revealed that the differences in agitation score between the temperament groups varied between the four flocks. The difference between the flocks in both production systems, but particularly between the Merino flocks, is suggestive of an environmental effect but this could be still underpinned by genetic differences either in the inherent reactivity of the ewes or because of sire effects. With regard to the latter, it needs to be remembered that whilst there is some linkage between flocks for the good and poor temperament sires, it is not completely balanced.

The important result to highlight was the overall mean difference in agitation score between the good and poor temperament groups. Although the magnitude of the group difference varied between the Merino flocks, genetic divergence in temperament has been achieved. The generation of these genetically divergent F1 Merino ewes provides an excellent resource to further test the hypothesis that temperament affects maternal behaviour and this in turn, will have a significant effect on lamb survival.

Another salient result here is the difference in agitation score between the sexes where ewes have a significantly higher score than rams. This is consistent with the results from a previous study where approximately 17,000 weaner sheep (wool, terminal sire and maternal flocks) were phenotyped for temperament using the IBT (Blache and Ferguson 2006).

The addition of weaner liveweight as a covariate was only significant for the Merino flocks.

Table 5 Least square means for the effect of flock, sex and temperament group on lamb survival

	IBT Agitation Score (units) Significance and LS means ± se
Merino (n=1735)	
Flock	*P<0.001*
Bilandri	$39.74^{a} \pm 2.88$
Merinotech	$38.84^{a} \pm 2.93$
Grindon	$67.00^{b} \pm 2.97$
Washpool	$68.33^{b} \pm 2.86$
Temperament group	*P<0.05*
Good	$46.31^{a} \pm 3.52$
Control	$51.66^{ab} \pm 5.06$
Poor	$62.48^{b} \pm 3.59$
Sex	*P<0.01*
Male	51.53 ± 2.50
Female	55.44 ± 2.51
Significant interactions	
Flock x Temperament group	*P<0.05*
Bilandri	
Good	$31.77^{a} \pm 4.54$
Control	$38.30^{a} \pm 5.45$
Poor	$49.18^{b} \pm 4.73$
Merinotech	
Good	38.19 ± 4.40
Control	35.34 ± 5.52
Poor	43.01 ± 4.47
Grindon	
Good	$59.20^{a} \pm 4.64$
Control	$65.87^{ab} \pm 5.61$
Poor	$75.95^{b} \pm 4.72$
Washpool	
Good	$56.10^{a} \pm 4.22$
Control	$67.13^{ab} \pm 5.45$
Poor	$81.78^{b} \pm 4.29$
Terminal Sire (n=1631)	
Flock	*P<0.001*
Genstock	$66.94^{a} \pm 3.10$
Gnaring Park	$26.18^{b} \pm 3.62$
Eagleridge	$61.67^{a} \pm 2.79$
Temperament group	*P=0.06*
Good	46.78 ± 3.76
Control	48.82 ± 5.72
Poor	59.19 ± 3.81
Sex	*P<0.001*
Male	49.13 ± 2.74
Female	54.06 ± 2.75

Least square means that do not share the same superscript are significantly different

(iv) **Sire solutions for IBT score**

Table 6 shows the sires used in the AHW.085 project, their EBVs for IBT agitation score (based on records collected as part of SHGEN.025 and AHW.140) and the sire solutions for IBT agitation score based on the progeny records from this project.

The correlation between the sire EBVs and sire solutions was 0.59 which is reasonably good given that the heritability of the trait is only 0.35.

Table 6 EBVs and sire solutions for IBT sore

Sire	Sire code	Breed	Temperament group	IBT EBV	EBV se	IBT Sire solution	se
20041050	1	M	Control	-	-	**-8.39**	**3.67**
1601852003030236	2	PD	Good	-20.57	19.7	**-11.23**	**4.19**
1618922002020555	3	PD	Poor	26.81	19.69	**-8.15**	**4.31**
1619722003030005	4	PD	Control	-	-	**-0.14**	**5.69**
1620022004040294	5	PD	Control	-	-	**-1.03**	**5.71**
1622882002020099	6	PD	Good	-25.33	25.33	**7.82**	**4.26**
1623682001010351	7	PD	Poor	13.63	12.46	**-6.75**	**4.14**
1640732002020284	8	PD	Poor	34.57	18.42	**11.09**	**4.20**
1640732002020433	9	PD	Good	-32.98	16.77	**-3.41**	**4.18**
2300262001012020	10	WS	Good	-30.24	22.51	**0.25**	**4.48**
2300992003030135	11	WS	Good	-34.34	17.64	**7.75**	**4.27**
2301132002020124	12	WS	Poor	20.72	25.71	**-0.44**	**4.28**
2301222001011131	13	WS	Poor	30.82	23.41	**4.24**	**4.85**
5003831999995873	14	M	Good	-30.51	18.04	**7.30**	**4.01**
5030541997970186	15	M	Poor	63.72	15.09	**18.61**	**3.76**
5030542001010228	16	M	Good	-38.41	14.72	**-10.56**	**4.04**
5030702002020150	17	M	Good	-30.02	19.64	**-0.51**	**4.49**
5032981998980034	18	M	Control	5.632	26.29	**18.60**	**3.56**
5049011999990075	19	M	Poor	18.87	26.71	**6.55**	**4.31**
6005711999990354	20	M	Poor	38.41	19.31	**6.39**	**4.31**
6009992000000189	21	M	Poor	32.91	21.27	**2.74**	**5.26**
6010822000001217	22	M	Poor	38.46	14.76	**1.73**	**5.20**
6012502001107351	23	M	Poor	51.82	17.07	**10.71**	**3.84**
6012502001107439	24	M	Good	-35.65	17.93	**-18.43**	**4.15**
6012502001107448	25	M	Good	-42.9	18.26	**-11.80**	**4.44**
501587199999J665	26	M	Good	-48.6	17.07	**-24.86**	**5.17**
5047671999OR0382	27	M	Good	-28.03	19.32	**-0.31**	**4.05**
5047672001VI0172	28	M	Poor	38.01	19.5	**8.31**	**4.07**
P240188	29	M	Control	-	-	**-6.08**	**3.60**

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Overall progress of the project

The project is still on schedule despite the minor delay finalising the DNA parentage assignment. The plans for the joining of the F1 ewes have ben revised. After discussions with MLA, there was agreement on two major changes to original plan. Firstly, only the good and poor temperament F1 ewes will be used. Semen from the control sires that were used in the first round of mating will be used to inseminate the F1 ewes. Secondly, the ewes from the three WA Merino flocks will be moved onto one property thus allowing greater control of their management.

Recommendations

There are no clear recommendations for industry that can be made at this juncture.

The results from Part A of the project do not indicate an association between temperament, maternal behaviour and lamb survival. However, it would be premature to draw this conclusion until the results from the F1 progeny are analysed in 2008/09. A revision to experimental protocol has been proposed and agreed. The results have also highlighted some important issues. It is well known that maintaining the condition of the ewe during pregnancy is absolutely paramount to maximising lamb survival. In hindsight, the ewes should have been offered more supplementation during pregnancy. The recommended changes in the experimental protocols will address this and therefore allow a more meaningful evaluation of the effects of temperament on lamb survival.

Annex 1

Timetable of the temperament testing and AI dates of the 7 collaborative farms involved in the project.

Collaborator	State	Temperament Testing	Synchronisation (CIDR Insertion)	AI
Billandri	WA	12-Dec-2005	29-Jan-2006	13-Feb-2006
Genstock	WA	20-Dec-2005	8-Feb-2006	23-Jan-2006
Grindon	WA	12-Jan-2006	4-Feb-2006	20-Feb-2006
Merinotech	WA	10-Jan-2006	17-Jan-2006	1-Feb-2006
Gnaring Park	WA	14-Dec-2005	19-Dec-2005	4-Jan-2006
Washpool	SA	14-Dec-2005	2-Jan-2006	20-Jan-2006
Eagleridge	VIC	17-Dec-2005	15-Jan-2006	2-Feb-2006

Annex 2 Summary of the pasture conditions, ewe supplementation and other lambing management practices for the seven flocks

Supplementation during pregnancy

Supplementation was carried out on all farms. Obviously pasture conditions varied significantly between farms and states. The Western Australian flocks (5 out of the 7 flocks included in the study) all had very little food on offer (FOO) in the period after scanning and indeed up to lambing. The supplementation offered is summarised below:

Grindon - 1.75 kg hd /wk of barley and minimal oaten chaff
Billandri - 2.625 kg hd /wk of a lupin/oats mix (25 %, 75 % respectively)
Genstock - *ad lib* silage (16% protein, 11 MJ/kg DM)
Merinotech - 0.4 kg hd /wk oats.
Gnaring Park - 4.9 kg hd /wk hay and 3.5 kg hd /wk oats

Washpool was in a similar situation, with all ewes being supplemented with silage and hay from 20th Feb until 2nd April at approximately 3.8kg hd/wk. After this twin bearers were fed at 8.5kg hd/wk, singles 1.2kg hd/wk. After 17 April twin bearing ewes were run into good quality phalaris paddock and no longer supplemented. Pasture quality improved and supplementation ceased for lambing.

Eaglerideg - ewes were separated after scanning with twins running in paddock with 1465 kgDM/ha and twins 2418 kgDM/ha. Prior to scanning all ewes were supplemented with 2kg hd/wk *Coprice* (14% protein, 12Mj ME).

FOO and supplementation during lambing

Grindon	FOO Singles 900 kgDM/ha, twins 1200 kgDM/ha Supplementation 1.05kg hd/wk sheep pellets.
Billandri	FOO Singles 400 kgDM/ha, twins 1000 kgDM/ha Supplementation Pea hay *ad libitum*, 2.6 kg hd/wk lupin/oats (25%/75%)
Genstock	FOO 1000 kgDM/ha (both singles and twins) Supplementation *Ad libitum* silage (as above), 1.05 kg hd/wk lupin/barley (33%/67%)
Merinotech	FOO 700 kgDM/ha (both singles and twins) 1.4 kg hd/wk sheep pellets.
Gnaring Park FOO	< 500kgDM/ha (both singles and twins) Supplementation 7 kg hd/wk hay,3.5 kg hd/wk oats (from 3 weeks prior to lambing)
Washpool	FOO Singles 1510 kgDM/ha twins 1672 kgDM/ha. Supplementation Nil
Eagleridge	FOO Singles 1286 kgDM/ha, twins 1969 kgDM/ha Supplementation *ad libitum* Coprice (14% protein, 12Mj ME).

Predator control, fox mitigation programs

Only 3 farms had any fox baiting programs, and of these only one (Eagleridge) was carried out prior to. Washpool was already locked into a baiting trial and was unable to deploy baits until after our lambing, however when they were deployed only two baits were taken.

Merinotech did deploy baits into one lambing paddock during lambing, with several baits taken.

Washpool, and I suspect many of the other farms as well, did have shooters on farm immediately prior to lambing, although no figures have been supplied.

9.2 B1 Draft manuscript

Genotype rather than non-genetic behavioural transmission determines the temperament of Merino lambs

S. Bickell[1], P. Poindron[2], R. Nowak[2], A. Chadwick[1], D. Ferguson[3] and D. Blache[1]

[1] School of Animal Biology, University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia
[2] Equipe Comportement, Neurobiologie, Adaptation, INRA, UMR85, Unité de la Reproduction et des Comportements, F-37380 Nouzilly ; CNRS UMR6175, F-37380 Nouzilly ; Université François Rabelais, F-37041 Tours, Haras Nationaux, F-37380 Nouzilly, France
[3]CSIRO Livestock Industries, Livestock Welfare, Locked Bag 1 Armidale, NSW 2350, Australia.

Running title: Genotype determination of temperament in lambs

Address for correspondence
Dr Dominique Blache
School of Animal Biology M085
Faculty of Natural & Agricultural Sciences,
The University of Western Australia,
35 Stirling Highway,
Crawley, WA 6009,
Australia

Tel: +61 8 6488 3587
Email: dbla@animals.uwa.edu.au

Abstract

Merino ewes have been selected, over 18 generations, for calm (C) or nervous (N) temperament using using an arena test and an isolation box test. We investigated the relative contributions of genotype versus the post-partum behaviour of their mother on the temperament of the lambs using a cross fostering procedure.

Forty eight multiparous calm and 52 nervous ewes were artificially inseminated with semen of a sire of the same temperament. At birth, 32 lambs of a given temperament line were cross fostered to ewes from the other line (16 NxC, 16 CxN), 34 lambs were cross fostered to ewes from the same line (15 CxC, 19 NxN) and 30 lambs were left with their birth mother (15 C, 15 N), to control for the effect of cross fostering. The temperament of the progeny was assessed at 2 occasions, 1 week after birth by measuring locomotor activity during an open field test and at weaning (16 weeks) by measuring locomotor activity during an arena test and agitation score measured during an isolation box test.

There was a genotype effect but no maternal or fostering effect on the lamb temperament at one-week. This may be because the maternal behaviour of the foster ewes did not differ considerably between the calm and nervous mothers during adoption or within the first week post partum. Similarly at weaning, only a genotype effect was found on the locomotor and agitation score. Therefore, it appears that temperament in Merino sheep is mainly determined by the genetic transmission of the trait across generations rather than behaviours learned from the mother.

Keywords: animal welfare, genetics, maternal behaviour, selection, sheep, temperament

Introduction

The process of animal domestication involves removing animals from their natural environment and placing them in a captive environment. How well animals adapt to their captive environment refers to the welfare of that individual (Broom, 1986). Animals that can successfully adapt to their environment are regarded as having a good level of welfare (Webster, 2005). Breeding animals that are capable of adapting to their environment is one way to improve the welfare of animals.

Sheep (*Ovis aries*), are a domesticated species that have not fully adapted to their captive environment. Sheep possess several unfavourable behavioural characteristics, such as extreme wariness and high reactivity to man and novel environments, which impedes their success of domestication (Hale, 1969, Price, 1984). Sheep are regularly exposed to man, novel environments and novel procedures in everyday life, which are physically and physiologically stressful for the individuals. Common stressors for sheep include shearing, handling, isolation, transport and yarding which often involves dogs, people and vehicles (Hargreaves and Hutson, 1990a, 1990b, 1990c, Corner *et al.*, 2006, Roussel *et al.*, 2006).

Selecting sheep that are better able to cope with the constraints of their captive environment can be achieved through selection for temperament. In Western Australia, there are Merino sheep that have been selected over 17 years for a low or high reactivity to humans and isolation at the University of Western Australia (UWA) (Blache and Ferguson, 2005b). Studies have shown that the two lines of sheep, respectively named calm and nervous, behave differently under various situations. Individuals with a calm temperament show reduced reactivity to man (Beausoleil *et al*., 2008), novelty (Bickell *et al.* 2008) and isolation (Blache and Ferguson, 2005a) compared with individuals with a nervous temperament. Such a reduction in reactivity to these everyday occurrences on farms means these animals are better suited to their domestic environment.

The transmission of a behavioural trait like temperament from one generation to the next, can occur through genetic and/or non-genetic behavioural transmission. While genetic selection of temperament or emotivity is possible, the emotional behaviour of mammalian and avian offspring is reported to be strongly influenced by the maternal behaviour of the mother (Anisman *et al.*, 1998, Caldji *et al.*, 1998, Caldji *et al.*, 2000, Richard-Yris *et al.*, 2004), thus suggesting a strong non-genetic behavioural transmission of the trait. Cross fostering studies with quail (Richard-Yris *et al*., 2004) and mice (Caldji *et al*., 2000) have demonstrated that the emotional reactivity of the young resembles more their adoptive mothers than their birth mothers. However, strong genetic effects have been found to affect the temperament of cross fostered rats (Broadhurst, 1961) and the behaviour of lambs (Boissy *et al*., 2005), which suggests a strong genetic transmission of the trait.
In the two lines of calm and nervous ewes selected at UWA, it has never been investigated whether the low reactivity to humans and isolation of the calm sheep results from the genetic transmission of the trait or from the direct influence of maternal care. A better understanding of the mechanisms involved in this type of selection will help increase the efficiency of selecting calm temperament sheep, and thus improve their welfare. Therefore the aim of this experiment was to investigate the relative contribution of genetic and non-genetic influence in the inheritance of temperament.

Materials and Methods

Experimental design

A cross fostering procedure was used in which lambs were removed from their mother immediately after birth and given to a foster ewe of either the same genetic line, or of the other one. In addition, two groups of calm and nervous lambs raised by their biological mothers were also studied to take into account possible effects of the fostering procedure itself. The behaviour of the ewe and lamb was monitored the following four hours to ensure that lambs had been fully adopted by the foster ewe. In addition, the ewe's behaviour was measured during a separation test conducted 48 hours after adoption to measure the ewe's distress reaction after being separated from her lamb. The temperament of the progeny was assessed at 2 occasions, firstly in the first week of life using an open field test and again at 1 week after weaning (16 weeks) using the standardised procedure developed by Murphy *et al.* (1994).

Animals

One hundred pregnant Merino ewes from the UWA temperament flock (48 calm, 52 nervous) were used (Murphy, 1999, Murphy *et al*. 1994). All ewes had been synchronised and were artificially inseminated on a single day by semen from a sire of the same temperament (calm or nervous). Each ewe had previously lambed in earlier years (multiparous) and was determined by ultrasound scanning to be carrying a single lamb. Three weeks before parturition ewes were transferred indoors to individual deep litter pens measuring 1.2 x 3.2 m. Ewes were fed hay and concentrates with water available ad libitum. On day 148 of gestation, parturition was induced using dexamethasone (16 mg / ewe injected IM) so that all births could be concentrated on a short period of time to facilitate fostering. Ewes were kept in indoor individual pens for one week after parturition, after which ewes and lambs were released into a paddock and kept together until weaning which occurred at 16 weeks post partum. The use of animals and the procedure were approved by the University of Western Australia Animal Ethics Committee (approval number 05100466).

Cross fostering procedure and behavioural observations

Each ewe was assisted at delivery, so that the lamb could be removed before the birth mother had any contact with it. The lamb of that ewe (ewe A) was put in a holding pen, ear tagged and weighed. As soon as another ewe of the desired line (ewe B) gave birth, her lamb was removed as well and she was immediately offered the neonate kept in the holding pen. Her own lamb was either put to foster with the previous ewe A, or kept until a ewe of the suitable line gave birth. If the delay between birth and fostering exceeded one hour, the lamb was returned to its birth mother's pen to stop the waning of the ewe's maternal behaviour but a 50 cm gap separated the ewe and lamb so that she did not form a selective bond with that lamb as demonstrated by Alexander et al. (1986). Lambs were allocated to one of the following groups: (i) 32 lambs of a given temperament line were cross fostered to ewes from the other line (16 N x C and 16 C x N), (ii) another 34 lambs were cross fostered to ewes from the same line (15 C x C and 19 N x N), while the remaining 30 lambs stayed with their birth dam as a control for the effect of fostering (15 C and 15 N). Immediately after the lamb was given to the foster ewe, a 5-minute behavioural observation was conducted to record the maternal acceptance or rejection behaviour of the foster ewe. Maternal acceptance behaviour included: number of low-pitched bleats and time spent licking the lamb. Rejection behaviour included: number of high-pitched bleats, butting or attempts to butt the lamb and circling away from the lamb. A lamb was considered to be adopted if the ewe showed maternal acceptance behaviour such as licking the lamb and low pitched bleats (Poindron et al., 1988). After this first session of observation, if the ewe showed signs of rejection behaviour such as circling away from the lamb or head butting, vaginal stimulation was performed to induce maternal behaviour (Keverne et al., 1983). The ewe's maternal acceptance and rejection behaviour was observed again after vaginal stimulation for another 5 minutes. Once the lamb was fully adopted, four other sessions of 5-minute observations were conducted each hour to follow the ewe-lamb interactions during the critical bonding period (Poindron et al., 1993). All the ewes received a foster lamb within 4 hours after giving birth.

Ewe-lamb separation-reunion test 48 h adoption

A ewe-lamb separation and reunion test was conducted 48 hours after adoption. All observations were made in the ewe's home pen. This test aimed at measuring the ewe's distress reaction after being separated from her lamb, while still being able to hear it. Each lamb was removed from its mother for 5 minutes and the behaviour of the ewe was directly recorded by an observer. The variables included: the number of low- and high-pitched bleats, the number of times the ewe circled the pen and the number of times she crossed the middle line of her pen. Then, the lamb was returned to its mother and the ewe's behaviour was observed again for another 5 minutes. The same variables were recorded again.

Behaviour of the lamb in an open field test at 1 week of age

All lambs from all treatment groups (46 born to calm ewes and 50 born to nervous ewes) were individually tested in an open field square arena for 5 minutes. Exposure to an open field arena is reported to elicit a repeatable emotional stress response in lambs under the challenge of social isolation (Moberg *et al.*, 1980, Kilgour *et al.*, 2006). The arena was 2.5 x 2.5 m and the sides were 1 m high and covered with Hessian (coarse jute cloth). The floor was divided into four squares of equal size. Immediately after being isolated from their mother, each lamb was put in the starting square in a standing position facing the centre of the arena. All the tests were videotaped to record the vocal and locomotor activities of the animals. The following variables were measured: number of bleats, number of squares crossed, number of jumps against the walls of the open field, latency to vocalize and jump.

Lamb temperament assessment at 16 weeks of age

All lambs from all treatment groups had their temperament assessed again at a week after weaning at 16 weeks of age in the standardised arena test and isolation box test developed by Murphy *et al.* (1994) and used for selecting the two temperament lines.

Arena test

This test evaluated the result of two conflicting motivations: social attractiveness towards flock-mates and avoidance of a motionless human. The testing arena (Beausoleil et al., 2008) consisted of a fully enclosed paved area that was 7m long, 3.3 m wide and 1.8 m high. The arena was divided into four equal areas. At one end of the arena was the holding pen containing four familiar flock-mates enclosed behind a panelled gate. In front of the holding pen a motionless human stood quietly for the entire duration of the test. Each animal was gently pushed into the arena through the entry gate and remained in the arena for 3 minutes. The number of areas crossed and number of bleats emitted were recorded.

Isolation box test

The isolation box test was performed immediately after the arena test. It consisted in measuring the agitation of each animal while it was isolated in an enclosed plywood box measuring 1.5 m x 1.5 m x 1.5 m high for 1 minute. Agitation was measured by an electronic meter that produced a numeric output that increased with the animal's movements and vocalizations (Blache and Ferguson, 2005b).

Statistical analyses

Maternal behaviour at fostering

Maternal behaviour was compared between calm and nervous foster and non-foster ewes after lamb introduction, after adoption and at 48 hours after adoption, using a three factorial (fostered, lamb genotype, maternal genotype) analysis of variance (ANOVA) including interactions with Genstat Eighth Edition (VSN International Ltd, Hemel Hempstead, United Kingdom). A square root transformation was used when data was not normally distributed. The proportions of calm and nervous foster ewes given vaginal stimulation after signs of lamb rejection were compared with a Chi Square test. Differences in the time from birth until the introduction of a foster lamb between calm and nervous foster ewes were analysed using a Kruskal-Wallis one way ANOVA.

Effect of the fostering procedure

The data from the cross fostered controls (calm lamb fostered to calm mother or nervous lamb fostered to nervous mother) and non fostered lambs were used to test the effect of the fostering procedure on the behaviour in the open field test, arena and the isolated box test using a general ANOVA including interactions. A square root transformation was used when data was not normally distributed. When data could not be normalized Mann Whitney tests were performed.

Effect of lamb and maternal genotype
The effects of lamb and maternal genotype on the behaviour in the open field test, arena and the isolated box test were analysed using a general ANOVA including interactions. As there was no effect of the fostering procedure only the data from the cross fostered animals were used in the analysis. A square root transformation was used when data was not normally distributed. When data could not be normalized, Mann Whitney tests were performed.

Results

Maternal behaviour during and after adoption
Eighty nine percent of the ewes readily accepted their foster lamb within the first 5 minutes of the adoption procedure. The 11% of ewes that showed signs of rejection successfully adopted their foster lambs after receiving 5 minutes of vaginal stimulation. The ratio of calm (12.9%) and nervous (9.6%) foster ewes that received vaginal stimulation did not differ ($\chi^2 = 0.16$; $P > 0.05$). The time it took for foster ewes to receive a foster lamb after birth did not differ (H = 0.002; $P = 0.95$) between calm (47.8 ± 10.7 min) and nervous ewes (41.2 ± 9.9 min).

The vocal behaviour of ewes differed according to the genotype of the foster lamb immediately after the lambs were first introduced to their foster mothers. Ewes with lambs of calm genotypes emitted more high-pitched bleats and less low-pitched bleats than ewes with lambs of nervous genotypes (Table 1). However, licking and maternal rejection behaviours like butting and circling away from the lamb did not differ between calm and nervous foster ewes or between ewes with calm or nervous foster lambs (Table 1). Once the lambs were fully adopted, the expression of maternal behaviour of calm and nervous ewes did not differ, regardless of the genotype of their foster lamb. The level of maternal acceptance behaviour and maternal rejection behaviour did not differ between groups (Table 1).

Forty eight hours after adoption, the circling behaviour of ewes differed according to their own genotype and their lamb's genotype (Table 2), when ewes were separated from their lambs for 5 minutes. Nervous ewes displayed more circling behaviour in the pen compared to calm ewes and ewes with nervous lambs also circled more than ewes with calm lambs (Table 2). However, no differences were detected in the number of times calm and nervous ewes crossed the middle line of their pen or in the number of high- or low-pitched bleats they emitted.

Once reunited with their lamb, calm and nervous ewes showed no differences in their vocal or locomotor behaviour (Table 2).

Table 1: Behaviour (mean ± s.e.m) of ewes towards their calm (C) or nervous (N) foster lamb during the adoption process and after the adoption process.

Lamb genotype	Calm	Nervous	Calm	Nervous
Maternal genotype	Nervous	Calm	Calm	Nervous
Group (Calm, Nervous)	**C-N**	**N-C**	**C-C**	**N-N**
ADOPTION				
Low pitched bleats	^{a} 27.1 ± 4.6	^{b} 39.9 ± 5.7	^{a} 24.6 ± 5.3	^{b} 39.9 ± 7.9
High pitched bleats	^{a} 8.9 ± 3.9	^{b} 3.9 ± 2.2	^{a} 7.1 ± 3.9	^{b} 2.0 ± 1.8
Time licking (min)	4.0 ± 0.3	3.8 ± 0.4	2.8 ± 0.5	4.0 ± 0.3
Rejection circling & butting	1.0 ± 0.6	0.6 ± 0.3	1.9 ± 1.0	1.0 ± 0.6
POST ADOPTION				
Low pitched bleats	11.4 ± 1.5	16.9 ± 2.3	14.9 ± 1.6	16.8 ± 3.0
High pitched bleats	3.1 ± 1.0	1.0 ± 0.4	1.0 ± 0.5	1.5 ± 0.8
Time licking (min)	1.4 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.2 ± 0.1
Rejection circling & butting	0.3 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.1

Row-means with different superscripts are significantly different ($P < 0.05$).

Table 2: Behaviour (mean ± s.e.m) of ewes towards their calm (C) or nervous (N) fostered or non fostered lamb during separation from their lamb for 5 mins and during the reunion with their lamb 48 hours after parturition.

	Non fostered		**Fostered**			
Lamb genotype	Calm	Nervous	Calm	Nervous	Calm	Nervous
Maternal genotype	Calm	Nervous	Calm	Nervous	Nervous	Calm
Group (Calm, Nervous)	**C-C**	**N-N**	**C-C**	**N-N**	**C-N**	**N-C**
SEPARATION						
High pitched bleats	25.5 ± 6.8	49.3 ± 9.4	28.1 ± 6.2	35.3 ± 5.2	38.8 ± 7.6	36.2 ± 4.8
Low pitched bleats	4.6 ± 1.8	2.5 ± 1.2	4.7 ± 2.2	3.8 ± 1.0	2.7 ± 0.9	5.8 ± 2.3
Crossing	3.0 ± 0.6	3.7 ± 0.9	3.4 ± 0.8	4.5 ± 0.8	5.1 ± 1.0	3.8 ± 0.7
Circling	^{ac} 0.2 ± 0.1	^{bd} 1.7 ± 0.4	^{ac} 0.7 ± 0.4	^{bd} 1.7 ± 0.4	^{ad} 1.4 ± 0.6	^{bc} 0.9 ± 0.2
REUNION						
High pitched bleats	0.7 ± 0.4	2.4 ± 1.0	0.5 ± 0.3	0.7 ± 0.3	1.2 ± 0.7	0.5 ± 0.2
Low pitched bleats	3.9 ± 1.1	5.1 ± 1.4	4.7 ± 0.8	4.8 ± 1.0	4.7 ± 1.6	6.4 ± 1.3
Crossing	1.2 ± 0.3	1.1 ± 0.4	0.9 ± 0.2	1.4 ± 0.4	0.6 ± 0.2	1.0 ± 0.3
Circling	0.5 ± 0.3	0.9 ± 0.5	0	0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.1

^{a} and ^{b}: comparisons to test the effect of the fostering procedure; ^{c} and ^{d}: comparisons to test the effect of lamb and maternal genotype; row-means with different superscripts are significantly different ($P < 0.05$).

Lamb temperament assessment at 1 week

There was no effect of the fostering procedure on the behaviour of the lambs in the open field test. Calm lambs with calm foster mothers showed similar locomotor and vocal behaviour to calm lambs with calm birth mothers, and nervous lambs with nervous foster mothers showed similar locomotor and vocal behaviour to nervous lambs with nervous birth mothers (Table 3).

In contrast there was a significant effect of the genotype of the lamb on the behaviour of the lambs in the open field test. Calm lambs bleated less but started bleating earlier, crossed less squares, jumped less and started jumping later than nervous lambs (Table 3).

Finally there was no effect of maternal genotype on the behaviour of the fostered lambs in the open field test. The number of jumps, bleats, movements, and the latency to start bleating and jumping did not differ between lambs with calm or nervous foster mothers (Table 3).

Table 3: Lamb behaviour (mean ± s.e.m) in an open field arena at 1-week of age. Lambs were either fostered or not fostered to calm (C) or nervous (N) ewes at birth.

	Non fostered		**Fostered**			
Lamb genotype	Calm	Nervous	Calm	Nervous	Calm	Nervous
Maternal genotype	Calm	Nervous	Calm	Nervous	Nervous	Calm
Group (Calm, Nervous)	**C-C**	**N-N**	**C-C**	**N-N**	**C-N**	**N-C**
Latency to bleat (s)	2 ± 0.5	2.5 ± 1.3	^{c} 1.5 ± 0.7	^{d} 2.4 ± 0.7	^{c} 0.7 ± 0.4	^{d} 2.3 ± 0.6
Number of bleats	^{a} 53.7 ± 4.2	^{b} 88.6 ± 6.5	^{ca} 62.5 ± 5.8	^{db} 85.4 ± 6	^{c} 61.9 ± 6.3	^{d} 78.9 ± 7.0
Latency to jump (s)	^{a} 167 ± 28	^{b} 16 ± 28	^{ca} 178 ± 34	^{db} 92± 22	^{c} 198 ± 34.3	^{d} 77 ± 22
Number of jumps	^{a} 4.8 ± 1.6	^{b} 6.4 ± 3.7	^{ca} 6.8 ± 2.5	^{db} 20.1 ± 4	^{c} 4.3 ± 2.0	^{d} 16.8 ± 3.6
Number of squares crossed	^{a} 16 ± 2.4	^{b} 6.3 ± 6.1	^{ca} 21.1 ± 3.5	^{db} 50 ± 5.3	^{c} 22.6 ± 4.3	^{d} 50.3 ± 6.5

^{a} and ^{b}: comparisons to test the effect of the fostering procedure; ^{c} and ^{d}: comparisons to test the effect of lamb and maternal genotype; row-means with different superscripts are significantly different ($P < 0.05$).

Lamb temperament assessment at 16 weeks of age

There was no effect of the fostering procedure on the behaviour of the lambs. Calm lambs with calm foster mothers showed similar locomotor and vocal behaviour to calm lambs with calm birth mothers in the arena test (Figure 1). Similarly, nervous lambs with nervous foster mothers also showed similar locomotor and vocal behaviour to nervous lambs with nervous birth mothers in the arena and in the isolation box test (Figure 1).
On the other hand there was a difference between the two genotypes on the behaviour of the lambs in the arena and in the isolation box test. Calm lambs did not cross as many squares in the arena and showed less agitation in the isolation box test than nervous lambs (Figure 1). No differences were detected in the number of bleats that calm and nervous lambs emitted in the arena test (Figure 1).

Figure 1: Lamb behaviour in the isolation box test (A) and arena test (B and C) at 16-weeks of age. Lambs were either fostered or not fostered to calm (C) or nervous (N) ewes at birth. Genotype of the lamb is indicated first. -> indicates the genotype of the foster mother. Different letters are significantly different ($P < 0.05$). a and b: comparisons to test the effect of the fostering procedure; c and d: comparisons to test the effect of lamb and maternal genotype.

There was no effect of maternal genotype on the behaviour of the fostered lambs in the tests. The number of bleats and movements in the arena test and the level of agitation in the isolation box test did not differ between lambs with calm or nervous foster mothers (Figure 1). In contrast there was an effect of the lamb's genotype on their behaviour in the arena and in the isolation box test. Regardless of the lambs foster mother's genotype, lambs with a calm genotype crossed less squares in the arena and showed less agitation in the isolation box test than lambs with a nervous genotype (Figure 1). No differences were detected in the number of bleats calm and nervous lambs emitted in the arena test (Figure 1).

Discussion

Genotype rather than non-genetic behavioural transmission determines the temperament of Merino lambs at weaning. The high heritability of lamb temperament may be the reason why Merino lamb temperament is mainly genetically transmitted and not influenced by postnatal behaviour of the mother. However, we can not completely rule out all maternal influences on the development of the offspring since the prenatal uterine environment can also affect the epigenetics of mammalian offspring. In addition, we also have to consider the possibility that lamb temperament was not influenced by ewe behaviour if the behaviour of the ewes did not differ between the two temperaments as was the case during the first few hours after parturition. However, it seems improbable that the behaviour of calm and nervous ewes did not differ while rearing their lambs since differences in ewe maternal and general behaviour have been reported in other studies (Putu, 1988, Murphy *et al.*, 1998, Blache and Ferguson, 2005a, Beausoleil *et al.*, 2008, Bickell *et al.*, 2008). Thus the temperament trait in Merino sheep has mainly a genetic mode of transmission which means the selection of Merino sheep, based on measures of temperament, has great potential in breeding programs.

It was predicted that the foster mother's temperament would influence the temperament of the lambs since an individual's temperament is not fixed at birth and can be significantly modified by the behaviour of the mother (Meaney 2001). Usually an individual's phenotype is the result of its own genotype plus the environmental effects experienced during development (Mousseau and Fox, 1998). The degree to which environmental factors, like a mother's behaviour, influence the expression of a trait would depend on the heritability of that trait. Trait heritability refers to the proportion of phenotypic variation that is due to the additive genetic effects (Glazier, 2002). Thus, traits with high heritability which have a high genetic component, would be less sensitive to environmental influences than traits with a lower heritability that have a lower genetic component. Maternal behaviour dramatically influenced the emotivity and maternal behaviour of cross fostered offspring of quail (Richard-Yris et al., 2005) and rats (Francis *et al*., 1999). While a predominance of genetic effects and weak maternal effects were found to affect the temperament of cross fostered Maudsley Reactive and Non Maudsley Reactive rats (Broadhurst, 1961) and the behaviour of lambs (Boissy et al., 2005).

The large variation in the degree to which the maternal environment affects an offspring's temperament is not surprising. Temperamental traits are variably influenced by maternal behaviour because they have moderate to high estimates of heritability (from 0.11 to 0.51 in sheep, cattle and dogs) (Mackenzie *et al.*, 1985, Murphy, 1999, Schmutz *et al.*, 2001). Temperament of Merino sheep, estimated using the isolation box test only, has been estimated to have a moderate heritability (0.2 to 0.4) (Blache and Ferguson, 2005a). Therefore since it has a moderate heritability it is less sensitive to environmental influences, and our results have shown that up until weaning, lamb temperament, expressed during a challenging situation, is not influenced by the behaviour of the mother.

Even though the postnatal behaviour of the ewe did not influence the temperament of Merino lambs we cannot rule out the possible effect of the maternal uterine environment on the development of the offspring. A mother's experience of her environment can be transmitted to her offspring in utero which may directly or indirectly influence offspring development (Mousseau and Fox, 1998). For example, stress in the pregnant dam can influence the development of the offspring through exposure of the fetus in utero to abnormally high concentrations of maternal glucocorticoids crossing the placental and blood-brain barrier (Zarrow *et al*., 1970, Barbazanges *et al*., 1996). Gestational stress can permanently alter the sensitivity of the offspring's HPA axis (Weinstock *et al*., 1998) thus impairing the stress-coping ability and predisposing the juvenile and adult offspring to be more sensitive to stressors (Braastad, 1998). In rats, differences between prenatally stressed and non-prenatally stressed rats are apparent when they are confronted with a novel or challenging situation (Weinstock 1997). Animals reared under extensive farming situations, like sheep in Australia, are often exposed to novelty, and thus the offspring of nervous ewes may have been exposed to higher levels of maternal glucocorticoids during gestation than the calm offspring. This may result in the offspring of nervous ewes being more sensitive to stressors than the offspring from calm ewes. Therefore the differences in the behaviour of the calm and nervous lambs may not only be due to genetic effects but also due to prenatal maternal effects.

Another possible explanation for no observable effect of the foster mother's behaviour on the temperament of the lambs may be because the behaviour of the foster mothers did not differ between the two genotypes during the first week post partum. Previous studies on the behaviour of calm and nervous ewes have shown that under experimental field conditions and in the presence of humans the two lines of sheep behave differently at the time of lambing. Calm ewes spend more time grooming their lambs, separate less often from their lambs during tagging and bleat more frequently to their young (Murphy *et al*., 1998). In the present study ewes were observed under indoor housing conditions, not under field conditions, and this may have influenced the ewe's expression of maternal behaviour. The cross fostering procedure required ewes to give birth in indoor individual pens and thus birthing conditions were optimal, contrary to the very unpredictable field conditions. The environmental conditions were stable, food and water were supplied ad libitum, predation risk was inexistent, there was no interference from other flock members, and humans were present everyday and associated with feeding. Dwyer and Lawrence (2000) have also found that when Suffolk and Blackface ewes, which are known to differ in their maternal behaviour, are housed indoors they do not show differences in the level of maternal behavior they display to their lambs.

However, the Merino ewes and their lambs in the present study were only kept indoors in individual pens for one week after parturition, after which the animals were released into a paddock. Under these extensive field conditions we can assume that calm and nervous ewes did express differences in maternal behaviour like those described by Murphy et al. (1998). In addition, the behaviour of calm and nervous ewes also differs in responses to stressful stimuli like man (Beausoleil *et al*., 2008), novelty (Bickell *et al*., 2008) and isolation (Blache and Ferguson, 2005a). In the present experiment the ewes were exposed once to a stressful situation; the separation from their lambs 48 hours after parturition. During this separation calm and nervous ewes differed in their circling behaviour which indicates that under challenging situations differences between the two temperaments become more apparent. The fact that other studies have shown that the behaviour of calm and nervous ewes does differ under extensive lambing conditions, and in response to stressful situations, alludes to the probability that the behaviour of the ewes did differ in our study. The experimental situation and observation methods used in the present work might not have been accurate enough to detect these differences.

Conclusions and animal welfare implications

Temperament traits related to social behaviour and response to human presence in sheep appear mainly determined by the genetic transmission of the trait across generations rather than by behaviours learned from the mother. Therefore this offers great potential to select sheep better adapted to standard farming procedures resulting in improvements to their welfare.

Acknowledgements

We would like to thank Céline Lenoury, Ken Hart, Baptiste Deleplace and Monique Poindron for all their help with the experimental work. We would also like to thank Steve Grey for all his help, advice and expertise and for caring for the animals. The work was supported by funding and a PhD scholarship from Meat and Livestock Australia and The University of Western Australia.

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S.L. Bickell[1]
R. Nowak[2,3,4,5]
P. Poindron[2,3,4,5]
F. Sèbe[1,2]
A. Chadwick[1]
D. Ferguson[6]
D. Blache[1]

[1]*School of Animal Biology-M085*
University of Western Australia
35 Stirling Hwy, Crawley
Faculty of Natural & Agricultural Sciences
Western Australia 6009, Australia
E-mail: dbla@animals.uwa.edu.au

[2]*INRA, UMR85 Physiologie de la Reproduction et des Comportements*
F-37380 Nouzilly, France

[3]*CNRS, UMR6175 Physiologie de la Reproduction et des Comportements*
F-37380 Nouzilly, France

[4]*Université François Rabelais de Tours*
F-37041 Tours, France

[5]*Haras Nationaux*
F-37380 Nouzilly, France

[6]*Livestock Welfare*
CSIRO Livestock Industries
FD McMaster Laboratories Chiswick
Locked Bag 1
Armidale NSW 2350 Australia

Temperament Does Not Affect the Overall Establishment of Mutual Preference Between the Mother and Her Young in Sheep Measured in a Choice Test

ABSTRACT: *Temperament influences maternal behavior and lamb survival in Merino sheep selected for calm or nervous temperament. The impact of this selection on mother-young recognition and early expression of temperament in lambs is unknown. We tested the ability of multiparous ewes selected for calm ($n = 16$) or nervous ($n = 18$) temperament to recognize their own lambs 6 hr after parturition, the ability of the lambs to display a preference for their own mother at 18 hr, and the temperament of the lambs at 1 and 16 weeks of age. Ewes and lambs from both genotypes showed a similar preference for their familiar kin. In contrast, differences in temperament were detectable at 1 and 16 weeks of age. Nervous lambs showed higher vocal and locomotor activity than calm lambs. Thus, temperament did not affect the early process of ewe-lamb bonding but might affect the quality of the mother-young relationship under more challenging situations.* © *2009 Wiley Periodicals, Inc.* Dev Psychobiol 51: 429–438, 2009.

Keywords: *choice test; ewe-lamb bond; open field test; recognition; sheep; temperament*

INTRODUCTION

Temperament traits participate in an individual's reproductive fitness in both human and non-human species (Wilson, Clark, Coleman, & Dearstyne, 1994), but the consequences a particular temperamental type has on survival and reproduction is unclear. It has been suggested that individuals with a highly reactive temperament may have an advantage in a natural context, where a highly reactive and cautious individual might be less susceptible to predation (Clarke & Boinski, 1995). In noncaptive bighorn sheep, the bold ewes have the advantage because they are less susceptible to predation than the shy ewes (Reale & Festa-Bianchet, 2003) and, in addition, they also have the advantage of reproducing earlier and having a higher weaning success than the shy ewes (Reale, Gallant, Leblanc, & Festa-Bianchet, 2000). In contrast, in farmed sheep, a low reactivity is a trait which improves reproduction because of a better expression of sexual behavior (Gelez, Lindsay, Blache, Martin, & Fabre-Nys, 2003) and maternal care (Murphy, Lindsay, & Le Neindre, 1998). In fact, in domesticated species, a low emotional reactivity seems to help the process of domestication (Price, 1984).

There is a large variability in the quantity and quality of maternal care expressed between and within breeds

Received 4 March 2009; Accepted 7 May 2009
Correspondence to: D. Blache
Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/dev.20382

(Dwyer & Lawrence, 2000). The differences in, for example, the amount of grooming behavior, responses to the lamb's sucking attempts, and the likelihood of desertion, are usually maintained over successive births, suggesting that they are intrinsic to the individual (Dwyer & Lawrence, 2000). Within-breed variation in maternal behavior has been observed in a ewe's reaction to their lambs being handled by a shepherd (O'Connor, Jay, Nicol, & Beatson, 1985), in the time they spend licking and grooming their lambs (Murphy, 1999) and in the time the ewes spend on the birth site (Putu, 1988). Ewes previously selected for their ability to rear lambs also show behavioral differences in an approach avoidance test, indicative of increased calmness (Kilgour & Szantar-Coddington, 1995; Murphy, Lindsay, & Purvis, 1994). These differences consistently displayed by individuals over successive births reflect their underlying temperament.

Studies with Merino ewes selected for a low or high reactivity to humans and isolation over 18 years, have shown that the two lines of sheep, respectively labeled "calm" and "nervous", behave differently at the time of lambing. Calm ewes spend more time grooming their lambs and emit more low-pitched bleats than nervous ewes (Murphy et al., 1998). Calm ewes also separate less often from their lambs, and for a shorter time, than nervous ewes. Because of these longer periods of separation and the lower level of grooming at parturition, nervous ewe and lambs would take longer to learn each others' characteristics, a factor known to influence lamb survival (Nowak & Poindron, 2006). In fact, the percentage of lambs dying between birth and weaning in the calm genotype is half of that of the nervous one, an observation made in both single- and twin-born lambs (Murphy et al., 1998). It was proposed that the higher rate of survival in lambs born from calm mothers was due to the establishment of a stronger bond between ewes and lambs in the calm than in the nervous animals (Murphy et al., 1998). Temperament could interfere with the process of mother-young recognition which is an essential component to the development of the early ewe-lamb interactions (Nowak & Poindron, 2006).

Temperament testing is generally conducted in adult animals because temperamental traits may change according to maturity and environmental experiences in early life (Van Reenen et al., 2004; Zuckerman, 1991). In humans, a temperament trait is defined as individual differences in behavior that are present early in life and are relatively stable both across various kinds of situations and time (Bates, 1987; Goldsmith et al., 1987). In Merino sheep, selection for a calm or nervous reactivity to humans and isolation has always been conducted at weaning (Murphy et al., 1998) but, irrespective of environmental influences, temperamental traits should be present early in life.

This present study aimed first to understand to what extent the selection for temperament influences the capacity of ewes and lambs to establish mutual preference in the first hours after parturition. We hypothesized that the development of such preferences will be stronger in the calm than in the nervous line. Based on results from previous studies (Keller et al., 2003; Terrazas et al., 1999), the ability of ewes and lambs to show a preference for each other was tested in a two-choice test at 6 and 18 hr after parturition, the dams being tested at 6 hr and the lambs at 18 hr, in order to detect potential differences between the two genotypes. Second, we hypothesized that the lamb would express the temperament of their parents early in life. In previous studies, the temperament has only been assessed in adult sheep, therefore we tested the lambs at 1 week of age and again at 16 weeks of age in order to detect differences in reactivity to social isolation according to their genetic line.

MATERIALS AND METHODS

Animals and Housing Conditions

Thirty-four pregnant Merino ewes (16 calm, 18 nervous) were selected from the temperament flock maintained at the Allandale Research Farm of the UWA School of Animal Biology, Wundowie. For selection purposes, the temperament had been tested in individual lambs when they were 3 months old, 2 weeks after weaning, using the two behavioral tests described by Murphy (1999) and Blache and Ferguson (2005). In brief, the first behavioral test is a choice test between approaching flock members and a human in an arena, the second test is an isolation test in a closed box. A score based on movements and vocalization was given to each individual. The animals used in this study were multiparous ewes randomly selected from the separate calm and nervous temperament lines. All ewes had been synchronized and had been artificially inseminated with semen of the best sire of the same temperament line. They had previously lambed and were all determined by ultrasound scanning to be carrying a single lamb. Three weeks before parturition ewes were transferred indoors to individual deep litter pens measuring $1.2\,m \times 3.2\,m$ and were fed hay and concentrates with water available ad libitum. The ewes and lambs remained in their individual pens until the temperament testing of the lambs at 1 week of age. After the testing they were all released back into the paddock. The use of animals and the procedure were approved by the University of Western Australia Animal Ethics Committee (approval number 05100466).

Recognition of the Lamb by Mothers 6 hr After Parturition

A testing pen was built in an isolated room situated 50 m away from the group of lambing ewes. The testing apparatus was a

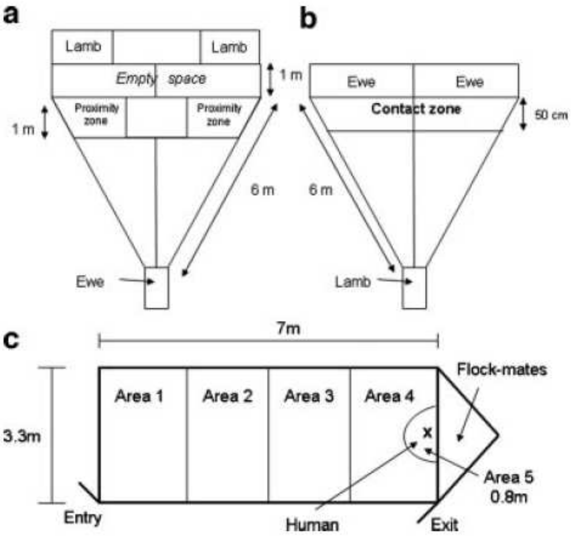

FIGURE 1 Schematic diagrams representing the testing pens used in the recognition of the young by the ewe (a), the preference for the mother by the lamb (b) and the arena used for temperament testing (c). In the test arena (c) the test sheep could move anywhere in areas 1–4 but was physically (but not visually) separated from the flock-mates by a metal barrier. The sides of the arena were 1.8 m high and were covered in shade cloth to create a visual barrier.

triangular pen (6 m × 6 m × 5 m) made of 1 m-high metal barriers (Fig. 1). The sides of the testing pen were covered with hessian to isolate the animal from external stimuli. The base of the triangle consisted of three pens side by side. Two of them contained the newborn lambs (1 m × 1 m) and were separated by an empty pen (3 m × 1 m). These three pens were separated from the testing area by a set of barriers, 1 m away, so that the ewes could see and hear the lambs but not smell them. This testing pen was designed from previous studies (Ferreira et al., 2000; Terrazas et al., 1999) showing that under such conditions, recognition of the lambs could not be based on olfactory cues. Olfactory recognition of the young by the ewe requires only 1/2 to 2 hr of contact (Keller, 2003) and there is evidence that recognition without the help of olfactory cues is established within 6/8 hr post-partum (Keller et al., 2003; Terrazas et al., 1999). Therefore the test was conducted 6 hr after parturition as our hypothesis was that it would be more challenging for the ewes from the nervous line and that they would not perform as well as calm ewes. The testing arena was divided into five zones and only an area of 2 m^2 in front of each lamb was considered as a zone of proximal contact between the ewe being tested and the stimulus lambs (Fig. 1).

Ewes and lambs were led to the testing area just before the time of the test. After the ewe was introduced into the waiting pen her lamb and an alien lamb from a previous birth were placed in their individual pens at the opposite corners of the testing arena. The position of the lambs was controlled so that in each line of ewes, half of the mothers were tested with their own lamb

765 on either the right or the left side of the arena. Before starting the test, the mother was restrained for 30 s in the waiting pen so that she could have the opportunity to see and hear both lambs before being released. The test started once the ewe was released and lasted for 5 min. The test was video-taped so that some variables could be analyzed thereafter. The following items were recorded from the test:

- Latency to reach a proximity zone.
- Latency to reach the proximity zone near own lamb.
- Total time spent in each proximity zone.
- Total number of visits to each proximity zone.
- Time spent looking at the lambs; looking being defined as the head axis of the ewe pointing towards a specific lamb (Terrazas et al., 1999; Ferreira et al., 2000). This was noted irrespective of the position of the ewe in the arena.
- The vocal activity of the ewe in each zone of the testing pen, her own lamb, and the alien lamb: number of high-pitched bleats (loud vocalization emitted with the mouth open) and number of low pitched bleats (soft vocalization emitted with the mouth closed, usually when mother and young are close to each other).

Preference for the Mother by 18-hr Old Lambs

Although there is no doubt that lambs can recognize their mother once they are 2 days old (Nowak, 1990), there is evidence that in the first day the orientation response depends mainly on the display of maternal acceptance by their own mother (Terrazas et al., 2002). Therefore the term preference was chosen instead of recognition in the study concerning lambs. The testing pen was derived from the one used in Experiment 1 (Fig. 1) and located in the same room. The only difference from the testing pen used in Experiment 1 was that the base of the triangle consisted of two pens side by side (3 m × 1 m) which contained the post-parturient ewes. The testing arena was divided into four zones and only a 50 cm-wide area in front of the pens containing the ewes was considered to be a zone of contact between them and the lamb. The bars of the pens left sufficient space to allow reciprocal nosing and sniffing between the ewes and the lamb, however the udder was out of reach. The expression of a preference for the mother was assessed in a two-choice test when lambs were 18-hr old. This was chosen as past studies had shown that single-born Merino lambs born outdoors developed a preference for their mother between 18 and 24 hr after birth (Nowak, Poindron, & Putu, 1989). As in the mothers, it was hypothesized that lambs born from the calm genotype would display better performance than lambs born from the nervous genotype, because of the challenging situation in which lambs had to make their choice.

At the time of the test the lamb was led to the testing arena with its mother. The mother of the lamb to be tested was placed in one of the two pens at the base of the testing arena and another ewe that had lambed at approximately the same time was placed in the other, without the lamb being able to see which pen its mother was placed in. The position of the ewes was controlled so that in each line of sheep, half of the lambs were tested with their mother on either the right or the left side of the arena. The lamb was placed in the starting pen, its head facing the two ewes. The test started once the lamb left its starting pen and lasted for 5 min. Each test was videotaped and analyzed later. The following items were recorded from the test:

- Latency to reach a contact zone.
- Latency to reach the contact zone near the own mother.
- Total time spent in each contact zone.
- Total number of visits to each contact zone.
- Vocal activity of the lamb, the mother, and the alien ewe: number of high-pitched bleats (loud vocalizations emitted with the mouth open) and number of low pitched bleats (soft vocalizations emitted with the mouth closed, usually when mother and young are close to each other).

The time lambs spent looking at the dams was not assessed because it was too difficult to correctly identify this behavior from the videotapes.

Temperament of Lambs

Temperament Testing at 1 Week of Age

Open field test. Thirty lambs of the temperament flock (14 born from calm ewes and 16 from nervous ewes) that were used in Experiments 1 and 2 had their temperament measured at 1 week of age in an open field arena. The open field test is a reliable method for eliciting a repeatable emotional stress response in lambs (Moberg, Anderson, & Underwood, 1980) as it measures an animal's level of fearfulness (Boissy, 1995).

Testing was carried out in a square 2.5 m × 2.5 m open field arena which was built in an isolated room situated 50 m away from the group of lambing ewes. The testing arena was formed by 1 m high metal barriers covered with hessian to restrict the lamb viewing outside the arena. The wooden floor was divided into four squares of equal size (numbered from 1 to 4) which were used to provide a measure of activity.

Each lamb was carried from its home pen and placed in the corner of square 1 in a standing position facing the center of the arena. Once released the behavior of the lamb was video recorded for the next 5 min. The following variables were analyzed:

- Number of high and low pitched bleats.
- Number of squares crossed and time spent in each square.
- Number of jumps.
- Latency to first vocalize, leave square 1 and start jumping.

At the end of each test the lamb was recaptured, carried to its home pen and placed with its dam.

Temperament Testing at 16 Weeks of Age Twenty-eight lambs of the temperament flock (14 born from calm ewes and 14 from nervous ewes) that were used in Experiments 1 and 2 had their temperament measured at 16 weeks of age in an arena test and in an isolation box test. An open field test (OFT) was used at 1 week of age, and not an arena test, because a conflict of motivation (arena test) in lambs would be redundant since lambs of 1 week of age do not have the great level of fear of humans, while they do at 16 weeks of age. In addition, previous experience can influence an individuals behavioral response (Webster, 2005), so at 16 weeks, the lambs were not tested in an OFT after the arena and isolation box test. These two tests were performed at 16 weeks because the lambs were from the temperament flock and they were required to have their temperament assessed according to the procedure used for the selection of the calm and nervous lines (Murphy et al., 1994; Murphy, 1999).

Arena test. The arena test evaluated the results of two conflicting motivations: social attractiveness towards flock-mates and avoidance of a motionless human. The testing arena (Fig. 1) consisted of a fully enclosed paved area that was 7 m long, 3.3 m wide and 1.8 m high. The arena was divided into five areas. At one end of the arena was the holding pen containing 4 familiar flock-mates enclosed behind a paneled gate. The flock-mates were randomly chosen and were of the same age as the sheep being tested. In front of the holding pen a motionless human stood quietly for the duration of the test. Each animal was gently pushed into the arena through the entry gate at the opposite end of the arena from the flock-mates and remained in the arena for 3 min. The number of areas crossed and the number of bleats emitted were recorded for each animal.

Isolation box test. The isolation box test involved the measurement of agitation of each individual animal while it was isolated in an enclosed plywood box measuring 1.5 m × 1.5 m × 1.5 m high for 1 min. Agitation was measured by an electronic "agitation meter" attached to the box that produced a numeric output based on vibrations made by the animal's movements and vocalizations (Murphy, 1999; Murphy et al., 1994).

Statistical Analysis

Recognition/Preference Tests For independent samples, the Mann–Whitney test for pair-wise comparisons was employed to detect differences between groups. For dependent samples, the Friedman test was used; this was followed by a Wilcoxon signed-ranks test for pair-wise comparisons after correction for multiple comparison by Bonferroni layering if need be. Box plots were chosen to characterize the data in the figures. Due to incomplete data recording because of technical problems with the recording equipment, 3 calm and 2 nervous lambs had to be excluded from the study (calm $n = 13$; nervous $n = 16$).

Open Field Test, Arena Test, and Isolation Box Test Latencies and number of jumps, bleats, squares crossed and time spent in each square were analyzed with a Mann–Whitney test. Due to incomplete data recording because of technical problems with the recording equipment, 2 calm lambs and 2 nervous lambs had to be excluded from the open field test (calm $n = 14$; nervous $n = 16$). Of these same animals tested in the open field test, 2 nervous lambs had died before testing in the arena and isolation box test (calm $n = 14$; nervous $n = 14$).

Comparison of Locomotor and Vocal Activity From 1 to 16 Weeks of Age Differences in the ranking of number of bleats and squares crossed (crosses) between weeks 1 and 16 were analyzed using the Mann–Whitney test and Spearman correlations were calculated between the two age groups. Box plots were chosen to characterize the ranked data in the figures.

Locomotor and vocal activities from calm and nervous lambs were pooled to determine if behaviors at 1 week of age remained stable through to 16 weeks of age using a Spearman's Correlation test. In all cases, p values <0.05 were considered as statistically significant.

RESULTS

Recognition of the Lamb by Mothers 6 hr After Parturition

All the ewes reached the zone of contact with the stimulus lambs before the end of the test.

There were few behavioral differences between calm and nervous ewes. Mothers from both genotypes did not differ in the time (in seconds) they took to initially reach a lamb (Calm $= 6 \pm 3$; Nervous $= 5 \pm 1$) or their own lamb (Calm $= 14 \pm 6$; Nervous $= 7 \pm 1$), in the number of visits (Own lamb: Calm $= 6.6 \pm 0.5$; Nervous $= 6.4 \pm 0.6$, Alien lamb: Calm $= 6.3 \pm 0.6$; Nervous $= 5.5 \pm 0.5$), nor in the time that they spent next to or looking at each lamb (Fig. 2a). However, calm mothers emitted significantly more low-pitched bleats than nervous mothers during the test (Calm $= 8.7 \pm 4.5$; Nervous $= 1.3 \pm 2.2$, $p = 0.007$).

Both calm and nervous ewes spent more time near their own lamb than near the alien during the first minute of the test (Fig. 2a), but the difference reached statistical significance only for the nervous ewes (Calm $p = 0.162$; Nervous $p = 0.043$). However, over the next 4 min of the test the time that mothers spent near their own lamb or near the alien lamb no longer differed (Fig. 2a).

Calm and nervous mothers spent significantly more time looking at their own lamb than at the alien lamb and this occurred not only during the first minute of the test (Calm $p = 0.055$; Nervous $p = 0.001$) but for the whole duration of the test (Calm $p = 0.048$; Nervous $p = 0.020$) (Fig. 2b).

Finally, the vocal activity of the stimulus lambs did not differ between the ewe's own lamb and the alien lamb.

Preference for the Mother by 18-hr Old Lambs

All the lambs reached the zone of contact with the stimulus ewes before the end of the test. While there was

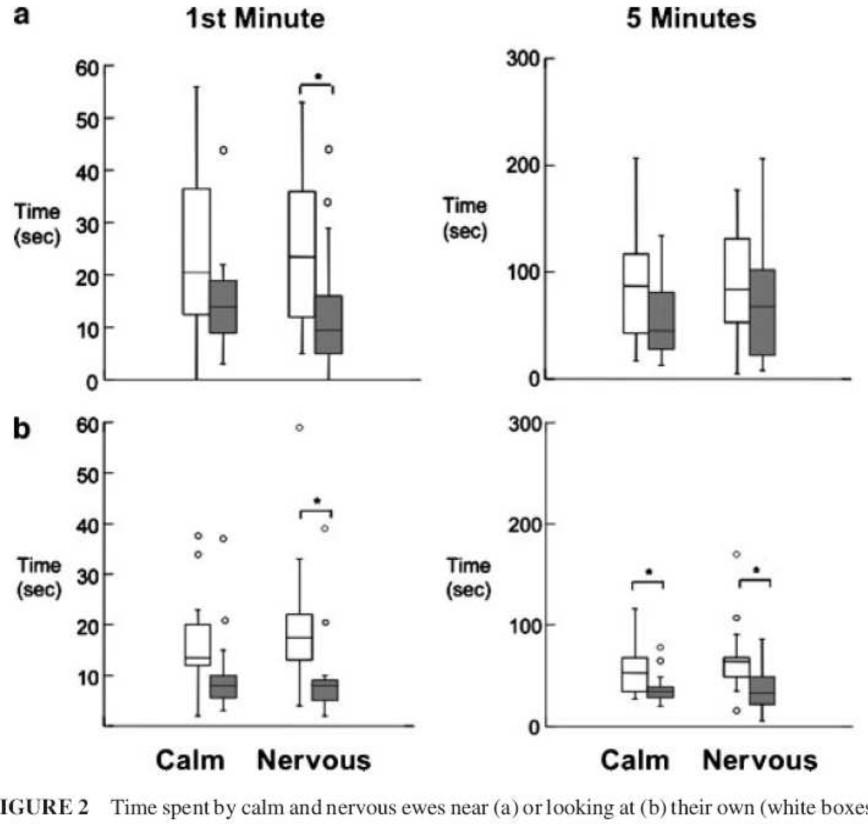

FIGURE 2 Time spent by calm and nervous ewes near (a) or looking at (b) their own (white boxes) or alien (gray boxes) lamb during the first minute or entire 5 min of the two choice test (calm $n = 16$; nervous $n = 18$). Values are medians (bar within the box) and upper and lower quartiles (boarders of box) with 10 and 90 percentile shown as the error bars. Outliers are shown as circles. $^*p < 0.05$.

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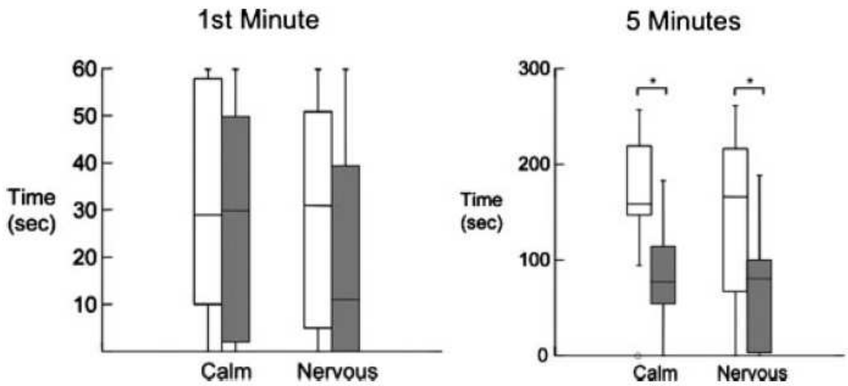

FIGURE 3 Time spent by lambs from the calm ($n = 13$) and the nervous temperament line ($n = 16$) near their own (white boxes) or alien (gray boxes) dams during the first minute or entire 5 min of the two-choice test. Values are medians (bar within the box) and upper and lower quartiles (boarders of box) with 10 and 90 percentile shown as the error bars. $^*p < 0.05$.

no difference in the time spent by lambs next to each mother during the first minute of the test, lambs of both genotypes spent more time next to their dam than next to the alien ewe over the whole duration of the test (Calm $p = 0.023$; Nervous $p = 0.05$, Fig. 3).

Whether the lambs were standing near their mother or the alien ewe, calm and nervous lambs spent a similar amount of time next to each dam (Fig. 3) and they did not differ in the number of visits made to each contact zone (Own ewe: Calm $= 3 \pm 0.5$; Nervous $= 3.4 \pm 0.6$, Alien ewe: Calm $= 3 \pm 0.5$; Nervous $= 2.8 \pm 0.6$). The time to reach their mother at the start of the test (Calm $= 95 \pm 21$; Nervous $= 85 \pm 23$) and the number of bleats (Calm $=$ 18.1 ± 5.5; Nervous $= 18.4 \pm 4.2$) did not differ either between the two genotypes.

The vocal activity of the stimulus ewes did not differ in the total number of bleats emitted (Calm $= 51.8 \pm 5.3$; Nervous $= 49.2 \pm 1.1$, $p = 0.539$) however, there was a trend for calm mothers to emit more low pitched bleats than nervous mothers (Calm $= 25.8 \pm 4.9$; Nervous $=$ 16.3 ± 1.1, $p = 0.10$).

Temperament of the Lambs

One Week of Age. Overall, in the open field test, nervous lambs ($n = 16$) had higher locomotor and vocal activity than calm lambs ($n = 14$). Nervous lambs were quicker than calm lambs to start jumping and trying to escape over the sides of the arena (Nervous $= 90 \pm 23.97$; Calm $=$ 197 ± 30.27, $p = 0.012$). Nervous genotype lambs also showed a tendency ($p = 0.075$) to leave the starting square sooner than calm genotype lambs. Throughout the entire 5-min test, nervous genotype lambs moved around more ($p < 0.001$), tried to escape more ($p = 0.009$) and emitted more high-pitched bleats ($p = 0.001$) than calm genotype lambs (Fig. 4). For all variables, these

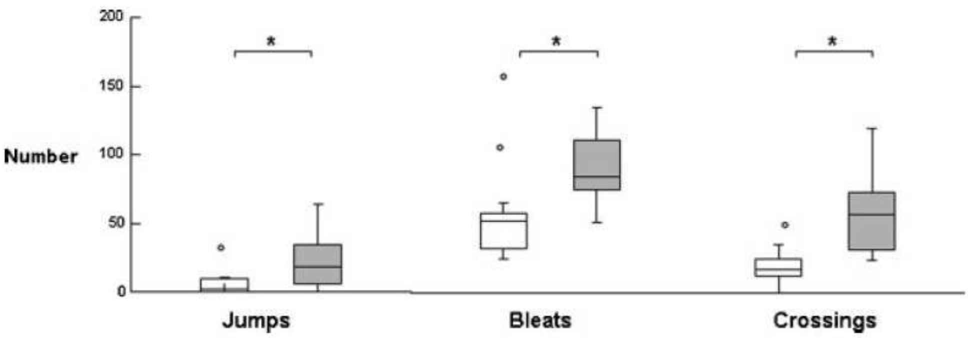

FIGURE 4 Number of jumps, bleats and squares crossed by 1-week-old lambs from the calm ($n = 14$; white boxes) and nervous ($n = 16$; gray boxes) temperament line in the 5 min open field test. Values are medians (bar within the box) and upper and lower quartiles (boarders of box) with 10 and 90 percentile shown as the error bars. Outliers are shown as circles. $^*p < 0.05$.

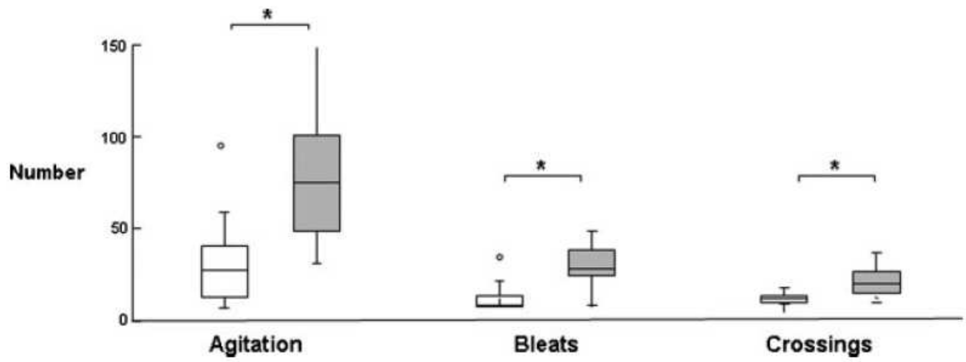

FIGURE 5 Agitation in the box test and number of squares crossed and bleats emitted in the arena test by 16-week-old calm ($n = 14$; white boxes) and nervous ($n = 14$; gray boxes) lambs. Values are medians (bar within the box) and upper and lower quartiles (boarders of box) with 10 and 90 percentile shown as the error bars. Outliers are shown as circles. $^*p < 0.05$.

differences were also significant in the first minute of the test and remained so for each 1 min interval (data not shown).

Sixteen Weeks of Age. Lambs from the nervous genotype ($n = 14$) showed higher locomotor and vocal activity than lambs from the calm genotype ($n = 14$) in the arena test. Nervous lambs crossed more squares ($p < 0.001$) and emitted more bleats ($p < 0.001$) than calm lambs (Fig. 5). In the isolation box test, nervous lambs showed higher levels of agitation ($p < 0.001$) than calm lambs (Fig. 5).

Comparison of Locomotor and Vocal Activity Between 1 and 16 Weeks of Age. Both the locomotor and vocal activity of calm and nervous lambs was highly correlated from 1 to 16 weeks of age. The number of bleats ($r^2 = 0.56$), jumps ($r^2 = 0.47$) and zones crossed ($r^2 = 0.68$) at 1 week of age in the open fiend test were positively correlated with the agitation score obtained in the box test at 16 weeks of age ($p < 0.001$). There was also a positive correlation between the number of bleats emitted in the open field test at 1 week and at in the arena test 16 weeks of age ($r^2 = 0.38$, $p < 0.05$). The number of zones crossed in the open field test at 1 week of age was correlated with the number of zones crossed in the arena test at 16 weeks of age ($r^2 = 0.49$, $p < 0.01$).

The ranked number of bleats emitted and the ranked number of squares crossed in an open field or arena test did not differ between the first and the16th week of age in calm and nervous lambs (Fig. 6). Both at 1 week in the open field test and at 16 weeks of age in the arena test, calm lambs emitted less bleats than nervous lambs (1 week: Calm $= 55.8 \pm 9.4$; Nervous $= 90.1 \pm 6.5$, 6.5, 16 weeks: Calm $= 4.7 \pm 2.0$; Nervous $= 54.2 \pm 6.9$, $p < 0.001$). Calm lambs also crossed less squares (1 week: Calm $= 19.6 \pm 3.4$; Nervous $= 53.9 \pm 7.0$, 16 weeks: Calm $= 7.3 \pm 0.7$; Nervous $= 34.5 \pm 4.7$, $p < 0.001$) in the open field and arena test than nervous lambs (Fig. 6). In addition, calm lambs also showed less agitation in the isolation box test than nervous lambs (Calm $= 31.2 \pm 5.9$; Nervous $= 78.4 \pm 9.3$, Fig. 6).

DISCUSSION

The present results did not support our hypothesis in favor of a better bonding process in the calm line compared to the nervous line. Not only did both calm and nervous mothers recognized their own lamb over the 5-min test but more unexpectedly nervous ewes seemed more prone to react positively in the initial stage of the test. Under field

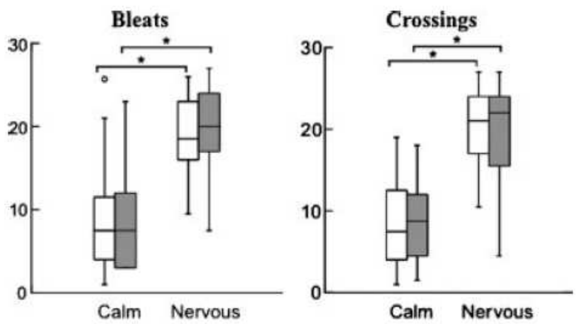

FIGURE 6 Ranking of the number of bleats and crossings displayed by lambs from the calm ($n = 14$) and nervous temperament line ($n = 15$) in the open-field tests conducted at 1 week of age (white boxes) and in the arena test at 16 weeks of age, (gray boxes). Values are medians (bar within the box) and first and third quartile ranges (boarders of box) with 10 and 90 percentile shown as the error bars. Outliers are shown as circles. $^*p < 0.05$.

conditions, calm ewes were reported to spend more time grooming their lambs, to emit more low-pitched bleats, to separate less often from their young and for a shorter time than nervous ewes (Murphy, 1999; Murphy et al., 1998). These behavioral differences and the higher mortality rate recorded in the nervous lambs led us to hypothesize that the bonding process was stronger in the calm temperament line. The present results show that this is not the case at least in the initial phase of the relationship, known to be crucial in terms of lamb survival, and under our experimental conditions. These unexpected results suggest that we must reconsider the significance of the behavioral differences between nervous and calm mothers at parturition and/or during the choice test. The explanation for the unexpected results could lie in a difference in the degree of alertness and/or in the memory processes between calm and nervous ewes. It is well recognized in animals and humans that attention and motivation co-vary with neuromodulatory systems of the brain to influence cognitive processes of attention, perception as well as the ability to retrieve memories or make new ones (Sarah, 2009). In addition, moderate stress or low doses of glucocorticoids usually enhance memory (de Quervain, Aerni, Schelling, & Roozendaal, 2009). Although the neurophysiological states of nervous and calm ewes around parturition still remains to be described, we know that nervous ewes exhibit greater alertness and higher release of cortisol in the presence of a noisy apparatus (Bickell, 2005) which are two stressful situations. The behavioral differences obtained by Murphy et al. (1998) under field conditions therefore could also be interpreted in terms of alertness. Because the divergent selection process includes the ewe's reaction to humans, the fact than nervous ewes emit less bleats, groom their lambs less often, or are separated more frequently from their young could just reflect the reaction to the human observer and not the quality of maternal care. In addition, should the birth process be considered as stressful an event to the mother as it is to the young (Lagercrantz, 1996), nervous ewes could be in a better physiological states to learn the characteristics of their young than calm ewes. The two-choice test combining mother-young separation, releasing the animals in a new environment, and the inability for partners to reunite properly, is a stressful situation. This is clearly shown in the level of agitation of the tested animals. Under such conditions, nervous mothers might display greater alertness or better discriminative ability than calm ones and be more prone to react to their lamb in the first minute of the test, performing slightly better 6 hr after parturition. It was not expected either that all lambs would show a preference for their mothers because Merino lambs are reported not to be very good at discriminating their mother before 24 hr of age (Nowak et al., 1989). This was not the case in the present work suggesting that lambs were not only rather quick at developing a preference for their dam but, in addition, the preference for the mother was not influenced by temperament. Testing the lambs at an earlier age could reveal a different outcome.

The lack of major differences in mutual bonding between calm and nervous animals cannot be explained by a lack of difference in the temperament of the lambs. In the open field test conducted at 1 week of age, nervous lambs showed higher locomotor and vocal activity than calm lambs. The same trends persisted until just after weaning, at 16 weeks of age, and variables are highly correlated between weeks 1 and 16. Open field or arena tests have been used in many studies to measure an animal's temperament [sheep: Gelez et al. (2003), cattle: Gauly, Mathiak, Hoffmann, Kraus, and Erhardt (2001), Horses: Hausberger, Bruderer, Le Scolan, and Pierre (2004), Mice: Gershenfeld, Neumann, Mathis, Li, and Paul (1997)], however, testing has usually been conducted after weaning or when the animals were adults. In our study the behavioral outcome of 1-week-old lambs in the open filed test is similar to the one at weaning in the arena test and in the box test. Although tested under different experimental situations, the correlation between the vocal and locomotor activities recorded at 15 weeks interval was positive and significant. In addition, lambs from the two lines showed consistent differences over time since animals from the nervous line showed higher vocal and locomotor activity than those from the calm line. Murphy et al. (1994) have shown that the component of temperament were highly repeatable when assessed at weaning, at 15 months and at 2.5 years of age. The present results demonstrate that, in sheep, temperament is set at a young age and traits such as calmness and nervousness can be characterized as early as at 1 week of age.

It seems that although certain dimensions of temperament affect the behavior of the ewe and lamb, these dimensions do not predict the overall establishment of mutual preference. As a consequence, since calm ewes and lambs do not establish mutual bonding more efficiently than nervous animals, then the cause of the higher infantile mortality in the nervous line still remains to be identified. It may not be specific to the first day after birth but spread over a period extending until weaning, in which case the causal factors will have multiple origins.

In the present study, the lambs were born indoors in small individual pens, the ewes were fed and watered ad libitum, and all the animals were accustomed to human presence. In the present experiment, individual confinement of ewes and lambs at lambing optimized contact between ewe and lamb thus favoring the expression of mutual ewe-lamb preference. In past studies, ewes gave birth outdoors under conditions that might have been less favorable for the formation of early bonding (Murphy, 1999; Nowak, 1990). The need to reunite with the flock after peripartum isolation, interference from flock mates, the need to graze or find water, fear of human, presence of predators or scavengers, can all affect the ewe-lamb bond and survival (Nowak, 1996). It is probably under more challenging situations than ours and over longer periods of time that calm ewes and lambs are likely to perform better than nervous individuals. This remains to be demonstrated. Recently a lot of attention has been devoted to the relationship between emotion and cognition in animals (Eisenberg, Fabes, Guthrie, & Reiser, 2000; Paul, Harding, & Mendl, 2005), where emotional states influence cognitive processes such as the appraisals of stimuli, events and situations. Our study highlights a crucial point: in the early steps leading to bonding, the slight differences in the cognitive ability of both lines cannot explain the effect of temperament on lamb survival.

We conclude that temperament alone does not affect significantly the establishment of mutual preference between the ewe and her newborn lamb even though there is evidence suggesting that certain dimensions of temperament are associated with infantile and maternal behavior such as proneness-to-distress during social separations (e.g., the open field test, the box test or the arena test) or alertness (two-choice test). Although these temperament dimensions did not predict the overall establishment of mutual preference under optimal lambing conditions (indoors) they might affect the strength of the ewe-lamb bond when it is established under more challenging conditions (outdoors).

NOTES

This research was supported by funding and a PhD scholarship from Meat and Livestock Australia and The University of Western Australia. We would also like to thank Steve Gray for all his help, advice and expertise and for caring for the animals.

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9.4 B2b Draft manuscript

Maternal behaviour and peripartum levels of oestradiol, progesterone and prolactin show little differences in Merino ewes selected for calm or nervous temperament under indoor housing conditions.

Submitted to Animal

S. Bickell[1], P. Poindron[2,3,4,5], R. Nowak[2,3,4,5], D. Ferguson[6], M. Blackberry[1] and D. Blache[1]

[1] *School of Animal Biology, University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia*

[2] *INRA, UMR85 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France*

[3] *CNRS, UMR6175 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France*

[4] *Université François Rabelais de Tours, F-37041 Tours, France*

[5] *Haras Nationaux, F-37380 Nouzilly, France*

[6] *CSIRO Livestock Industries, Livestock Welfare, Locked Bag 1 Armidale, NSW 2350, Australia.*

Corresponding author: Dominique Blache. E-mail: dbla@animals.uwa.edu.au

Running head: Ewe temperament, maternal behaviour and hormones

Abstract

Temperament influences the expression of maternal behaviour in sheep under experimental field conditions. Whether maternal behaviour between ewes selected for a calm or nervous temperament is independent from environmental conditions remains to be investigated. In addition, the level of maternal behaviour expressed by mothers is correlated with the concentration of hormones during the peripartum period. Therefore we investigated if the selection for temperament had resulted in hormonal differences between the two lines concerning the hormones that could be involved in the onset of maternal behaviour. Oestradiol, progesterone and cortisol concentrations from 4 days before parturition through to 24 hours after parturition were determined from blood samples collected from 10 calm and 12 nervous ewes. Behavioural interactions between ewe and lamb were also recorded for 2 hours starting at parturition. Mothers of both temperament lines showed adequate maternal behaviour under our controlled conditions of study. Therefore, our results do not suggest that selection for a calm or nervous temperament has profoundly affected the intrinsic ability of mothers to display adequate maternal behaviour. The hormonal differences between the two temperament lines were generally small and their possible influence on the display of maternal behaviour would remain to be demonstrated.

Keywords: ewe, temperament, maternal behaviour, hormones

Implications:

Lamb survival is affected by the selection on temperament and temperament influences the expression of maternal behaviour in sheep under experimental field conditions. Our study investigated whether the level of maternal behaviour expressed by mothers is correlated with the concentration of hormones required for the onset of maternal behaviour. We found that the hormonal differences between the two temperament lines were generally small and thus our results do not suggest that selection for a calm or nervous temperament has profoundly affected the intrinsic ability of mothers to display adequate maternal behaviour under indoor housing conditions.

Introduction

A lamb's survival depends, in part, on the quality of maternal care it receives from its dam. Lamb survival is affected by the selection on temperament. In Merino ewes selected for their reactivity to humans and isolation (Murphy 1999; Murphy *et al.* 1994) the survival rate of lambs born from calm ewes (calm lambs) is double that of the lambs born from nervous ewes (nervous lambs) (Murphy 1999). Results by Murphy (Murphy 1999; Murphy *et al.* 1994) suggest that this is due to some differences in the expression of maternal behaviour between the two temperament lines during the early postpartum period. Calm ewes were found to have higher levels of maternal behaviour (grooming and bleating) than nervous ewes (Murphy 1999). However, the behaviour of the ewes was watched by human observers standing 1 meter away from the ewe and lamb. The animals are selected on their reactivity to humans and thus the presence of the human during the observations may have affected the behaviour of the ewes. Therefore whether the differences in the maternal behaviour between the ewes selected for a calm or nervous temperament is independent from the environmental conditions remains to be investigated.

The spontaneous onset of maternal behaviour in the ewe at parturition is largely under hormonal control (Poindron and Levy 1990), even though the degree and duration of maternal behaviour each animal exhibits at parturition varies between individuals. Past studies have found that the level of maternal behaviour expressed by dams is correlated with the concentration of hormones during the peripartum period (Dwyer *et al.* 2004; Pryce *et al.* 1988). Therefore, the quality of maternal behaviour expressed by individuals could be due to the level of circulating hormones required for the onset of maternal behaviour. The differences in maternal behaviour reported by Murphy *et al.* (1994, 1999) between the two temperament lines could be due to some differences in the level of hormones required for the onset of maternal behaviour.

In sheep, as in other mammals, oestradiol plays a critical role in the facilitation of maternal behaviour. Oestradiol plays a priming role and is critical for vaginal stimulation to be able to trigger maternal behaviour (Kendrick and Keverne 1991; Meurisse *et al.* 2005; Poindron *et al.* 1988). In addition, oestradiol is positively correlated with the quality of maternal behaviour displayed by the ewe. Higher levels of maternal care (licking and grooming, udder acceptance) were found in Blackface ewes that also had higher oestradiol concentrations immediately before birth (Dwyer *et al.* 2004). Other hormones have also been reported to come into play in the control of maternal behaviour at parturition in mammals. Progesterone may be inhibitory if present at birth, but it can also facilitate the action of oestradiol (Kendrick *et al.* 1997). Corticosteroids also have been reported to play a role in the modulation of ongoing maternal behaviour during the post-partum period (Rees *et al.* 2004) however, very high levels due to stress may be detrimental to maternal behaviour (Tu *et al.* 2005; Weinstock 2005).

The aims of this study were 1) to verify if the maternal behaviour of the two temperament lines differed in controlled conditions in which the animals had been intensely habituated to the presence of humans, in particular by daily feeding and 2) to investigate if selection had resulted in hormonal differences between the two lines concerning the hormones that could be involved in the onset of maternal behaviour.

Materials and Methods

Experimental design

Twenty-two single bearing multiparous Merino ewes from a flock of sheep selected for their calm or nervous temperament (10 calm, 12 nervous) were transferred indoors and kept in individual pens two weeks before parturition. Blood samples were collected at 6 hour intervals from all ewes from 4 days before parturition through to 24 hours after parturition. Behavioural interactions between ewe and lamb were recorded for 2 hours starting at parturition. Oestradiol, progesterone and cortisol concentrations in each blood sample were determined by radioimmunoassay. The use of animals and the procedure were approved by the University of Western Australia Animal Ethics Committee (approval number 05100466).

Animals

The 22 experimental ewes (10 calm, 12 nervous) came from the temperament flock maintained at the Allandale Research Farm of the UWA School of Animal Biology, Wundowie, Western Australia. The two temperament lines of ewes resulted from selection for 17 generations on the basis of testing temperament in individual lambs when they were three months old, two weeks after weaning, using a combination of two behavioural tests as described by Murphy *et al.* (1994). In brief, the first behavioural test is a conflict of motivation test between approaching flock members and a human in an arena, the second test is an isolation test in a closed box. A score based on movements and vocalisation and combining the behaviour of the subject in the two tests was given to each individual. All ewes were synchronised in estrus using progesterone sponges and naturally mated with a sire of the same temperament line. They had previously lambed and were all determined by ultrasound scanning to be carrying a single lamb. Two weeks before parturition ewes were transferred indoors to individual deep litter pens measuring 1.2 x 3.2 m. and were fed hay and concentrates with water available *ad libitum*. The animals were habituated to the presence of humans by twice daily feeding for two weeks.

Blood sampling procedure

Blood samples, approximately 9 ml, were collected from the jugular vein at 6 hr intervals from all ewes from 7 days before the expected date of birth until the first signs of parturition. Only samples from the last 4 days of pregnancy were available for all ewes and thus analyses were only performed on samples from 4 days before parturition. No sampling was taken during labour. Immediately after parturition a blood sample was taken and then samples were taken at 1 hr, 2 hr, 8 hr, 16 hr and 24 hours after parturition. Blood samples were centrifuged immediately after sampling and the resultant plasma was separated and stored at -80˚C until assayed for oestradiol, progesterone, prolactin and cortisol.

Lambing procedure

Ewes lambed without assistance as far as possible. However, if the ewe had begun labour but had not delivered the lamb after it had been visible for 1 hr then assistance was given (Dwyer *et al.* 2004).

Behavioural observations

Maternal and lamb behaviour data were collected by live observations. Behaviours of the ewe included licking, low-pitched and high-pitched bleating, acceptance and rejection at the udder, backing and circling away. Behaviour of the lamb included latency of attempts to stand and suck as well as latency to actually stand and suck. The definitions of behaviours recorded are given in Table 1.

Radioimmunoassay

Oestradiol was measured in a single assay after extraction with diethyl ether. The limit of detection was 0.7 pg/ml and the intra-assay coefficients of variation was 3.9%, 2.1% and 5.1% at 48.7, 13.1 and 2.56 pg/ml.
Progesterone samples were assayed using Progesterone DSL-3900 radioimmunoassay kit (Diagnostic Systems Laboratories, Webster, Texas). The limit of detection was 0.15 ng/ml and the intra-assay coefficients of variation was 2.6% and 3.0% at 1.01 and 10.21 ng/ml.
Cortisol samples were assayed using GammaCoat Cortisol ^{125}I radioimmunoassay kit (DiaSorin, Stillwater, Minnesota). The limit of detection was 3.5 nmol/L and the intra-assay coefficients of variation was 1.2% and 4.0% at 43.1 and 109.6 nmol/L.

Statistical analyses

Maternal behaviours were compared between temperament lines using a two factorial (temperament line, time after birth) analysis of variance (ANOVA) with Genstat Eighth Edition (VSN International Ltd, Hemel Hempstead, United Kingdom). The first and second hour after birth were analysed separately to determine if the wane in maternal behaviour over time differed between the two temperament lines. Differences between temperament lines on the ratio of the number of times the ewe terminates the sucking bout / time the lamb suckles at the udder were analysed with 2 sample t-tests. Differences between temperament lines on the proportion of ewes circling and backing away from their lambs were carried out with a Fisher's Exact probability test (Genstat Eighth Edition). Differences between temperament lines in the duration of labour and time to shed placenta were analysed with 2 sample t-tests. Lamb behaviours were compared between temperament lines using a Mann-Whitney U test.
Hormone concentrations were analysed with repeated measures ANOVA in three separate time periods (1) 4 days to 1 day before parturition, (2) immediately after birth, 1hr after, 2 hrs after and (3) 8, 16 and 24 hrs after birth. Post-hoc comparisons were performed with Least Squares Means Differences. Hormone concentrations before parturition were averaged for each day. Comparisons between the end of a time period and the beginning of the next were analysed using *t* tests for paired samples.

Results

Parturition and shedding of placenta

The duration of labour did not differ between calm (13.5 ± 2.3 min) and nervous (19.4 ± 7.3 min) ewes (p = 0.44). Likewise, the time it took to shed the placenta after giving birth did not differ between calm (197.3 ± 24.3 min) and nervous (208 ± 14.0 min) ewes (p = 0.707).

Maternal behaviour

All but one ewe began licking their lamb within the first 10 min of birth. There was no effect of temperament on the maternal behaviour of the ewe during the first hour after birth. Calm

and nervous ewes did not differ in the amount of time they spent licking their lamb, or in the number of low-pitched bleats they emitted (Table 2). However, nervous ewes tended ($p = 0.06$) to emit more high-pitched bleats than calm ewes (Table 2). The ratio of ewes that terminated sucking bouts / time at the udder and the proportion of ewes that circled and backed away from the lamb also did not differ between the two temperament lines (Table 2). Aggressive behaviour towards the lamb, such as head butting or head threats, was not observed in any of the two temperament lines.
Calm and nervous ewes did differ in their expression of maternal behaviour during the second hour after birth. Calm ewes spent longer licking their lambs and emitted less high-pitched bleats than nervous ewes (Table 2). There was also a lower proportion of calm ewes that circled away from their lamb compared with nervous ewes ($p = 0.027$; Table 2). On the other hand, the number of low-pitched bleats the ewes emitted did not differ between calm and nervous ewes and there was no difference in the ratio of ewes that terminated sucking bouts / time at the udder or in the proportion of ewes backing away from their lamb between the two lines (Table 2).

When the observations of the 2 hours post partum are pooled, calm and nervous ewes differed in the number of high-pitched bleats and the proportion of ewes that circled away from their lamb. Calm ewes emitted less high-pitched bleats and circled away from their lamb less compared with nervous ewes (Table 2). Otherwise, calm and nervous ewes did not differ in the amount of time they spent licking their lamb, in the number of low-pitched bleats they emitted or in the ratio of ewes that terminated sucking bouts / time at the udder or in the proportion of ewes that back away from the lamb (Table 2).

Lamb behaviour

There was no difference between temperament lines on the latency of the lambs to start bleating after birth or in the latency to get upright to their knees (Table 3). Calm and nervous lambs did differ in the latency to suckle at the udder where nervous lambs were quicker to start suckling than calm lambs (Table 3). Nervous lambs also tended to be quicker at attempting to stand ($p = 0.063$) as well as at standing ($p = 0.072$) compared with calm lambs (Table 3). However, calm and nervous lambs did not differ in the amount of time they spent suckling at the udder during the first (Calm = 3.51 ± 1 min, Nervous = 5.6 ± 1.2 min; $p = 0.218$), second (Calm = 4.87 ± 1.2 min, Nervous = 5.56 ± 0.6 min; $p = 0.497$) or whole 2 hours (Calm = 8.38 ± 1.76 min, Nervous = 11.15 ± 1.5 min; $p = 0.234$) postpartum.

Comparison of hormone concentrations before, during and after parturition between the two temperament lines.

In both temperament lines oestradiol concentrations steadily increased over the 4 days before parturition and up to the sample just after parturition. Then, these concentrations declined in both lines up to 24 hours postpartum. Differences between lines were found only one day before birth where the nervous ewes had significantly higher levels of oestradiol compared with the calm ewes ($p = 0.016$; Figure 1) and 24 hours after birth, where oestradiol levels were higher in calm than nervous ewes ($p = 0.006$; Figure 1).

Plasma progesterone steadily declined before parturition in both temperament lines ($p < 0.001$; Figure 1) and remained generally low after parturition. No significant differences in concentrations were found between temperament lines before parturition. On the other hand, concentrations immediately after birth were higher in the calm ewes than the nervous ewes ($p = 0.022$).

Cortisol concentrations increased during the 4 days before parturition and then peaked immediately after birth in both temperament lines ($p < 0.01$; Figure 1). One hour after birth cortisol levels declined in both temperament lines, however, from 2 hours after birth to 8 hours after birth, cortisol concentration decreased in the calm ($p = 0.04$) ewes but not in the nervous ($p = 0.5$). There was also a tendency ($p = 0.11$) for nervous ewes to have higher cortisol levels than calm ewes at 8, 16 and 24 hours after birth (Figure 1).

Discussion

Mothers of both temperament lines showed adequate maternal behaviour under our controlled conditions of study. In both lines, all ewes displayed the full complements of maternal behaviour to be expected at parturition: licking of the neonate, acceptance at the udder, emission of maternal bleats, while showing no signs of aggression towards their neonate. Therefore, our results do not suggest that selection for a calm or nervous temperament has profoundly affected the intrinsic ability of mothers to display adequate maternal behaviour, at least on a qualitative basis. Similarly, the behaviour of the lamb did not seem to be strongly affected by selection, the only difference being that lambs of the nervous line were slightly more active than calm lambs and reached the udder sooner than calm lambs. Finally, concerning hormones, the differences between lines were generally small and, their possible influence on the display of maternal behaviour would remain to be demonstrated. Thus, it is well established that estradiol facilitates maternal behaviour and that its levels are positively correlated with maternal care (Dwyer *et al.* 2004; Pryce *et al.* 1988). Nonetheless, in the present study estradiol levels differed significantly between the two lines only on the last day prepartum and these levels were in fact higher in the nervous line of mothers that tended to display quantitatively less maternal care (i.e. duration of licking time during the second hour and higher percentage of mothers terminating suckling). Therefore, taken together, our results do not suggest that selection of sheep on their calm or nervous temperament has resulted in intrinsic differences in the quality of maternal behaviour expressed in controlled housing conditions or its immediate hormonal control by estradiol between the two lines. Hence, the poorer maternal behaviour of nervous ewes and the ensuing higher lamb mortality reported in that line by Murphy *et al.* (1994, 1999) under outdoor lambing conditions cannot be directly accounted for by an intrinsically poorer maternal behaviour of nervous mothers.

The apparent discrepancy between the small differences of maternal behaviour found in the present study and those reported by Murphy (1999) is likely to be due to the differences in experimental conditions between the two studies. In our study, possible interactions between the display of maternal behaviour in each line and other factors were kept to a minimum: animals gave birth indoors, in individual pens and with sufficient food to meet their requirements. In addition, they had been extensively habituated to human presence, which was associated with the reward of food distribution. In contrast, in the studies of Murphy *et al.* (1994, 1999), animals were kept outside, were exposed to winter climatic conditions, had to spend a large amount of time grazing to meet their metabolic requirement and had been conditioned to human presence only by habituation without any reward and with the possibility to keep at a distance from humans at any time. It is likely that at the time of maternal behaviour observation at lambing, this has resulted in two extremely different situations for the ewes. In the present study, mothers had been used to positive reinforcing of human presence and impossibility to escape, whereas in the studies of Murphy *et al.* (1994, 1999), mothers were facing a conflict between taking care of their neonate and escaping a human being closer than usual. Thus in the field, this latter situation could have resulted in a higher frequency of nervous mothers showing disturbance of maternal behaviour or even desertion of their lamb, especially in case of adverse climatic condition or reduced food availability. In other words, inadequate maternal care may not have been the result of poor maternal behaviour *per se*, but because of its inhibition by a situation perceived as more stressful (human presence + adverse environmental factors) in nervous females. Similar results have been reported by Dwyer and Lawrence (2000) who found that Suffolk and Blackface ewes do not show differences in the level of maternal behaviour they display to their lambs when they are housed indoors, despites the fact that they are known to differ in their maternal behaviour. In other words in the case of our two temperament lines, nervous temperament could impair the display of maternal behaviour because of a higher distress response of mothers to any disturbing or stressing factor. In addition, the tendency of nervous mothers to circle away from their lambs more often than calm ewes reported here may also be increased in a stressful situation.

The behaviour of the lamb may be another factor that contributed to the poorer performances of the nervous line of sheep in Murphy's studies (Murphy *et al.* 1994, Murphy 1999). Here, we found that nervous lambs were more active than calm lambs. This is in agreement with the fact that lambs of the nervous line show stronger locomotor and vocal responses in an open-field test soon after birth (Bickell *et al.* 2009). Again, in undisturbed conditions, this higher reactivity is probably not a disadvantage for nervous lambs, as they appear to suckle earlier than calm lambs. On the other hand, in case of a challenging situation in the field, a higher reactivity in the lamb is likely to impair further its capacity to reunite with its dam in case of disturbance, thus adding to the inadequate response of the dam. This may have been an additional factor exacerbating the poorer performance of nervous mothers in the studies of Murphy, despite the habituation procedure experienced by the ewes before lambing.

Two other components of maternal behaviour – grooming duration during the second hour postpartum and frequency of bleats - differed between calm and nervous ewes in our study. Whether these differences are important for lamb survival in the field is not clear. Calm ewes groomed their lambs more than nervous ewes during the second hour post partum. However, it must be noted that overall licking time did not differ between the two lines, and the difference found during the second hour postpartum may well be the result of the difference of behaviour of the lambs in the two lines. Nervous lambs were more active from the start and reached the udder sooner than calm lambs, and therefore activity of calm lambs was likely to increase during the second hour and stimulate the grooming behaviour of the mother at that time. This is supported by the fact that the suckling activity of calm lambs was only 63% that of nervous lambs in the first hour postpartum, whereas it represented 88% of it in the second hour. This may have resulted in reinforcing the behaviour of calm mothers. This agrees also with the findings of Dwyer and Lawrence (1999) that during the second hour post partum, Suffolk lambs received more grooming attention than Blackface lambs, presumably because Suffolk lambs were less likely to have sucked.

Concerning vocal behaviour, nervous ewes bleated consistently more than calm ewes in the current study, mainly because of a higher number of high-pitched bleats. On the other hand, the frequency of low-pitched bleats did not differ between the two lines. Low-pitched bleats are used for communication between a ewe and her lamb at short distance (Shillito 1972) while high-pitched bleats can indicate signs of distress (Dwyer *et al.* 1998; Kiley 1972). Therefore, the lack of difference in low-pitched bleat frequencies emitted by calm and nervous mothers could indicate that the maternal behaviour did not differ between the two lines. Nonetheless, the higher frequency of high-pitched bleats in nervous mothers may suggest that nervous ewes were more stressed by the lambing process. This is also congruent with the fact that a high rate of high-pitched bleats contributes to a higher index in the selection scheme (Murphy *et al.* 1994) and with the higher vocal activity of sheep of the nervous line in open-field tests (Bickell *et al.* 2009; Nowak *et al.* 2006). Therefore, the higher vocal activity of nervous mothers is probably a general reflexion of their nervous temperament rather than some specific difference of maternal behaviour with calm ewes. Furthermore, recent studies have shown that even high-pitched bleats are part of the normal repertoire of postparturient ewes and that vocal communication, both through low- and high-pitched bleats, is beneficial to the mother-young bond (Sèbe *et al.* 2009; Sèbe *et al.* 2007) and that emission of high-pitched bleats tend to increase after parturition (Dwyer *et al.* 1998). Therefore, in any case, the difference of vocal activity encountered here between the two temperament lines of mothers is unlikely to contribute to the poorer lamb survival found in nervous ewes.

The comparison of hormonal profiles in the mothers of the two temperament lines does not suggest that the levels of estradiol, the main peripheral hormone involved in the facilitation of maternal behaviour in sheep (Lévy 1996.; Poindron *et al.* 1988), have been strongly affected by selection. Differences between the two lines remained small even when they were significant and the difference was not consistent over time, levels being higher in nervous ewes the day before lambing, but higher in calm ewes after lambing. At the time when estradiol levels could have influenced the display of maternal behaviour during the period of observation (i.e. before parturition or just after lambing), estradiol levels either did not differ between the two lines, or were higher in the nervous line of ewes. Therefore, the differences in maternal behaviour observed in the present study about licking or vocalisations cannot be explained by differences in estradiol levels, which correlate positively with maternal behaviour (Dwyer *et al.* 2004; Pryce *et al.* 1988).

The same applies for progesterone, which can prepare the action of estradiol (Kendrick *et al.* 1997), or even for the ratio estradiol/progesterone, which reflects the change of balance between these two hormones and may influence the onset of lactation (Martinet and Houdebine 1999) or maternal behaviour in some species (Fleming *et al.* 1997; Pryce 1996). However, it cannot be ruled out that other factors influencing colostrum and milk production differ between the two lines and may influence survival. In fact, colostrum quality differs between the two temperament lines (Hart *et al.* 2006; Williman 2006) and colostrum is important for the initial attachment of the newborn lamb to its mother (Nowak 2006).

Interestingly, the only consistent difference between calm and nervous mothers that did emerge from our study of hormones profiles concerned cortisol. This hormone, which is a good indicator of stress in sheep (Hargreaves and Hutson 1990; Roussel *et al.* 2006) started to differ in the sampling following the shedding of the placenta. While concentration of cortisol kept decreasing in calm mothers, they did not do so up to 24 hr postpartum in nervous mothers. This may suggest that shedding the placenta was a more disturbing experience for the nervous ewes than the calm ewes. This possibility is supported by the fact that a disturbing effect of placenta shedding is easily observed in females giving birth for the first time. In such females, it is common to see mothers that keep circling for considerable time, as if trying to “escape” the stimulation of the hanging placenta, until it is shed (Poindron, personal communication). Therefore the higher levels of cortisol found in the nervous mothers from 8 hrs onward may reflect their higher responsiveness to stress. This is also consistent with the facts that the neurobiological response to stress is coordinated in part by the HPA axis (Tyrka *et al.* 2006) and that in several species the selection for temperament can affect the individuals HPA axis sensitivity (Curley *et al.* 2008; Kosten and Ambrosio 2002; Landgraf *et al.* 1999). In addition to a stressing effect of placenta shedding, another non-exclusive explanation of the higher levels of cortisol found in nervous ewes would be that this difference depends on a response to another type of stress, the metabolic and/or behavioural challenge associated with the establishment of lactation and maternal care. For example, preliminary work in our laboratory suggests that partitioning of energy may differ between our two temperament lines of sheep, especially in twin bearing ewes under nutritional restriction (Williman 2006). This difference in energy partitioning may explain why ewes of the calm line produce better quality colostrum (Hart *et al.* 2006; Williman 2006) and an equal quantity of milk that contains more protein (Sart *et al.* 2004) than ewes of the nervous line. Similarly, the shift from a neuroendocrine control of maternal behaviour dependent on maternal physiology at parturition to a neurosensory control dependent on sensory interactions with the young (Poindron *et al.* 2007) may be more challenging in mothers of the nervous line than in calm mothers. Investigating the existence of any differences in the control of the HPA axis between the two lines and their consequences would be necessary to confirm this possibility. Similarly, it would be necessary to investigate any differences in the physiological control of lactation between the two temperament lines that could contribute to differences in lamb survival.

In conclusion, the higher lamb mortality and poorer maternal behaviour reported in a 3 year study by Murphy *et al.* (1994) in the line of sheep with a nervous temperament compared with the line with a calm temperament is unlikely to result directly from poorer maternal or lamb behaviour in the nervous line. Rather, the lower performances found in the nervous line are more likely the result of some negative interaction between the nervous temperament and behaviour of the mother and/or the young in outdoor lambing conditions. In addition, the higher mortality found in the nervous line may involve some physiological factors directly or indirectly related to the degree of nervousness of the animals that still remain to be identified based on the similarities of performance between lines under indoor housing conditions.

Acknowledgements
This research was supported by funding and a PhD scholarship from Meat and Livestock Australia and The University of Western Australia. We would also like to thank Steve Gray for all his help, advice and expertise and for caring for the animals.

Figure 1: Plasma concentrations of (a) oestradiol, (b) progesterone and (c) cortisol from 10 calm (open circle) or 12 nervous (closed circle) ewes from 4 days before parturition through to 24 hours after parturition. Standard errors are shown as bars. *: $p < 0.05$, #: $p = 0.1$.

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Figure 1

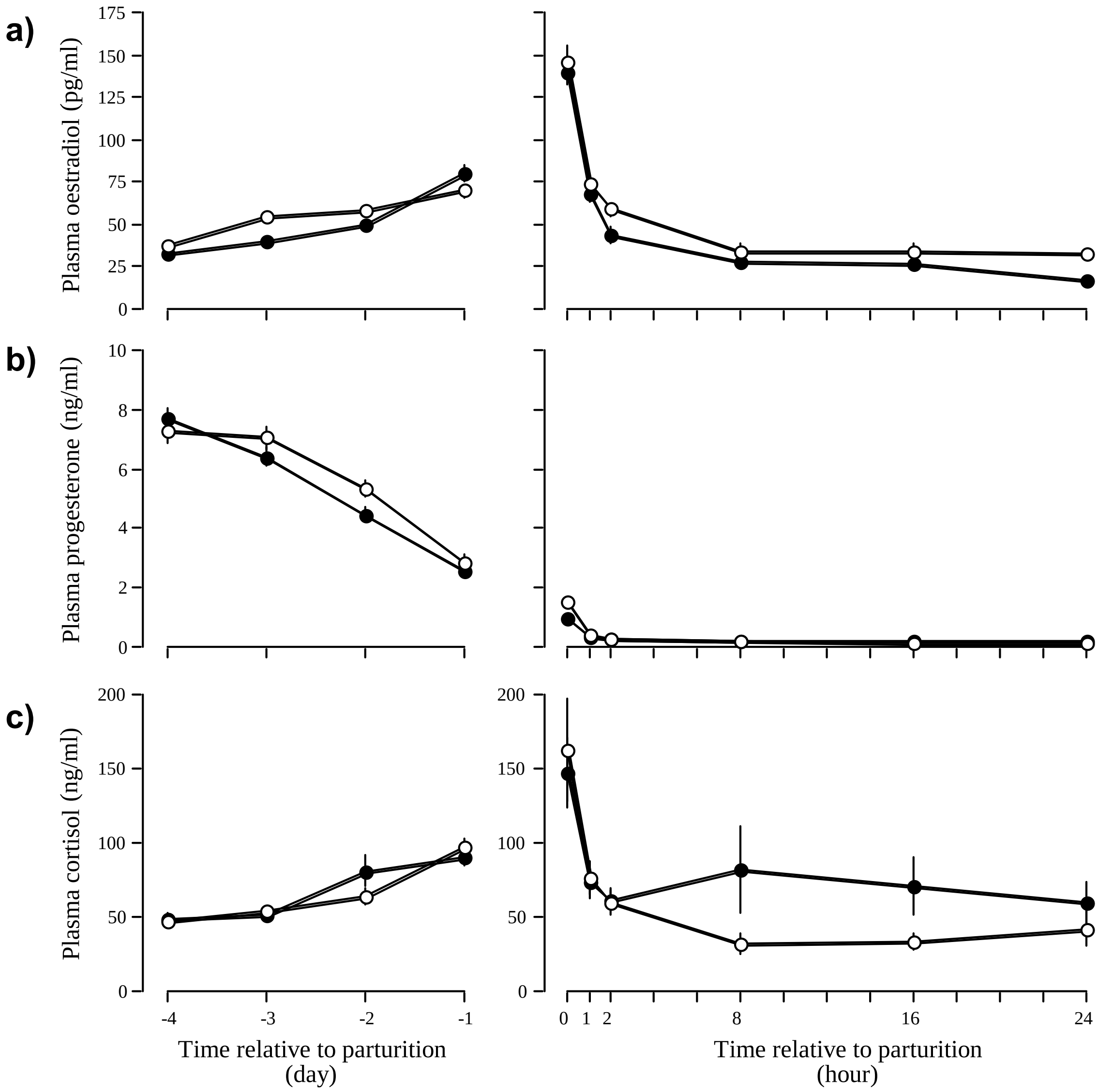
a)
Plasma oestradiol (pg/ml)
175
150
125
100
75
50
25
0
b)
Plasma progesterone (ng/ml)
10
8
6
4
2
0
c)
Plasma cortisol (ng/ml)
200
150
100
50
0
-4
-3
-2
-1
Time relative to parturition
(day)
200
150
100
50
0
0 1 2
8
16
24
Time relative to parturition
(hour)

Table 1: Definitions of maternal and neonatal behaviours.

Behaviour	Description
Maternal behaviour	
Licking	Licking of the lamb.
Low pitched bleat	A low pitched 'rumble' or 'mmm' bleat made with the mouth closed.
High pitched bleat	Louder 'baa' bleat made with the mouth open.
Terminating sucking bout	Ewe moves away from the lamb while lamb is at the udder
Circling	Ewe steps sideways, moving hindquarters away from the lamb when it goes toward the inguinal region.
Backing	Ewe steps backwards as lamb moves forwards toward the inguinal region.
Head butt	Ewe's head makes contact with the lamb following a fast movement of head
Head threat	Ewe makes a fast movement of head as if to head butt lamb, but does not make contact
Lamb behaviour	
Suckles at the udder	Lamb has teat in its mouth for at least 5 s.
To knees	Lamb on chest, pushes up on knees, supporting part of body off the ground.
Attempts to stand	Lambs stands on all 4 feet for less than 5 s.
Stands	Lamb stands on all 4 feet for more than 5 s.

Table 2: Maternal behaviour of calm and nervous ewes during the first and second hour post-partum.

	1st hour			2nd hour			Total 2 hours		
Behaviour	Calm	Nervous	p	Calm	Nervous	p	Calm	Nervous	p
Number of ewes	10	12		10	12		10	12	
Duration of licking (min)	35.1 ± 3.5	35.4 ± 4.0	0.542	23.8 ± 1.8	16.2 ± 2.2	0.018	58.8 ± 3.9	53.9 ± 4.0	0.398
No. low pitched bleats	399 ± 62	461 ± 67	0.209	218 ± 49	292 ± 39	0.181	617± 104	808 ± 87	0.17
No. high pitched bleats	19 ± 7	93 ± 35	0.064	25 ± 11	122 ± 30	0.015	43 ± 17	219 ± 62	0.02
Termination of sucking bout / time at udder	0.15	0.3	0.369	0.32	0.77	0.2	0.82	0.53	0.355
% of ewes circling away from lamb	20	50	0.204	30	83	0.027	40	83	0.07
% of ewes backing away from lamb	20	25	1	10	25	0.59	20	33	0.64

Table 3: Behaviour of calm and nervous lambs immediately after birth.

Behaviour	Calm	Nervous	U stat	p value
Latency to bleat (s)	19.5 ± 16.8	9.1 ± 4.8	26	0.60
Latency to suckle at the udder (s)	56 ± 10.9	34.3 ± 6.4	30	0.05
Latency to knees (s)	12.9 ± 3.4	12.3 ± 2.6	54	0.97
Latency to attempt to stand (s)	23.1 ± 5.2	10.3 ± 1.2	28.5	0.06
Latency to stand (s)	36.2 ± 8.4	19.9 ± 3.6	24.5	0.07

9.5 B2c – Hart et al (2006). Aust. J. of Exper. Agric. 46:827-829.

www.publish.csiro.au/journals/ajea *Australian Journal of Experimental Agriculture*, 2006, **46**, 827–829

Colostrum quality of ewes of calm temperament is not responsible for low lamb mortality

K. W. Hart[A,B,D], *A. Chadwick*[A], *F. Sebe*[C], *P. Poindron*[C], *R. Nowak*[C] *and D. Blache*[A]

[A]School of Animal Biology, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.
[B]Department of Agriculture Western Australia, 10 Dore St, Katanning, WA 6317, Australia.
[C]UMR 6175 INRA-CNRS-Université de Tours-Haras Nationaux, INRA, 37 380 Nouzilly, France.
[D]Corresponding author. Email: khart@agric.wa.gov.au

Abstract. Perinatal lamb mortality is a serious issue for the sheep industries. Starvation is implicated in many of these lamb deaths. Ewes with a calm temperament lose significantly fewer lambs than ewes with nervous temperament, particularly in the critical first 3 days after birth. Colostrum provides essential nutrition to neonatal lambs. This research set out to determine whether ewe temperament affects the quantity and quality of colostrum that ewes produce. Calm temperament was found to have an influence on the viscosity of colostrum 6 h after birth. High variability in all aspects of colostrum production were found in the study and may be a limiting factor in lamb survival in certain circumstances. There is little evidence, however, to suggest a strong association with ewe temperament and is therefore unlikely to explain the lower lamb mortality that is reported to occur among calm ewes.

Introduction

Lamb mortality has a serious impact on the efficiency of sheep production but is also becoming an increasingly important animal welfare issue for the sheep industries. Major sheep producing countries report that an average of 15 to 30 percent of all lambs die before they are weaned (Aspin 1997; DEFRA 2005; Mason and Bactawar 2003; Neary 1996). Up to 80% of lamb deaths occur within 3 days of birth (Moule 1954; Watson 1972). Most perinatal deaths are caused by starvation due to inadequate energy intake (Alexander 1984). Colostrum provides essential nourishment to the perinatal lamb and also contributes to rapid bonding to the mother (Goursaud and Nowak 1999). Both of these factors are essential to the lamb's survival. However, there is a wide range in both the quality and quantity of colostrum produced by different ewes (Banchero *et al*. 2004*a*, 2004*b*; Murphy 1999; Murphy *et al*. 1996).

Another factor that influences early lamb survival is the temperament of the mother. Ewes with calm temperament wean significantly more lambs than nervous ewes. This is largely attributable to a lower mortality rate during the critical first 3 days after parturition (Murphy 1999; Murphy *et al.* 1996). While it is understood that both factors are implicated in neonatal lamb survival it is not known whether these factors are implicated with or independent of each other. If ewe temperament does influence colostrum production then it is possible that ewes with calm temperament produce colostrum that is of better quality and/or quantity than colostrum produced by nervous ewes. To investigate this hypothesis we compared the production of colostrum in 2 lines of Merino sheep that have been selected for calm or nervous temperament over the past 15 years.

Material and methods

This project is conducted with approval from the University of Western Australia's Animal Ethics Committee. The approval number is RA/3/100/466.

Two treatment groups were established using the temperament selection lines from the University of Western Australia's Allandale flock. Due to a limit on the number of pens available for indoor housing, 25 calm and 25 nervous, 4- to 6-year-old ewes were initially selected. Selection criteria included that the ewes were identified by ultrasound scanning as having a single lamb. The ewes were multiparous and had previously successfully reared a lamb. For various reasons including still-born and twin lambs only 19 ewes from the nervous line and 15 from the calm line were able to be included in the study.

When the ewes were moved indoors, about 1 week prior to lambing, the mean weight of the calm line was 57.7 ± 1.28 kg and the nervous line was 56.1 ± 1.31 kg. The ewes were fed a pelleted ration (late pregnant ewe cubes), which contained 12% protein and 10.5 MJ/kg ME.

During lambing, the ewes were in single pens and under 24 h observation. Each lamb was weighed at birth. The ewe was then given an intramuscular injection containing 1 i.u. of oxytocin. The ewe was then milked from the right mammary and the colostrum produced was weighed. An assumption was made that both sides of the udder have similar volumes of colostrum, thus allowing us to estimate total volume at each milking. A score for viscosity was given using the subjective scale described by McCance and Alexander (1959; Table 1). Viscosity scores measure the fluidness of the colostrum. Lower scores indicate that a sample has a less fluid state. A sample of about 20 mL was frozen after adding 50 µL of potassium dichromate. The lamb was not permitted access to the right mammary between sampling periods. This was done by modifying a pair of panty hose to form a

Table 1. Classification of scores for types of secretion expressible from the udder (from McCance and Alexander 1959)

Type	Description
0	No secretion expressible
1	Clear serous straw-coloured liquid
2	Similar to 1, but higher viscosity
3	Clear to opaque yellow liquid of very high viscosity
4	Opaque yellow liquid of moderate viscosity
5	Opaque yellow to cream coloured liquid, slightly more viscous than normal ewes milk
6	Similar to normal ewes milk, but cream coloured
7	Opaque white liquid similar to normal ewes milk

harness and tying it to the ewe. This routine was repeated at 1, 3, 6, and 10 h post-partum. The lamb was allowed *ad libitum* access to the left mammary.

The frozen samples of colostrum were thawed slowly, over 4 days. The proportion of fat, protein and lactose in fresh colostrum was determined for each sample, using a Milkoscan 133 (Foss Electric, Hillerød, Denmark).

The lambs were weighed at birth, 4 days and 8 days of age (Table 2). At 2 days of age the ewe and lamb were moved from individual pens to larger group pens. At 8 days of age the ewe and lamb were moved outdoors into a paddock.

The main effect of temperament and its interaction with time were tested for each colostrum variable using a mixed model (REML) analysis with repeated measurements. A comparison of the means for each trait at each sample time was also made using a homoscedastic *t*-test. Colostrum viscosity was analysed using Mann–Whitney U Test.

Results

The colostrum of nervous ewes was more viscous at each sample time but only significantly so at 6 h postpartum (d.f. = 32, $t_{0.025}$, ≤2.080; Table 3). In contrast, the milk fat content tended to be higher in the nervous ewes than in calm ewes but was only significant at 10 h postpartum (d.f. = 32, $t_{0.05}$, ≤1.720; Table 4). The proportion of lactose was greater in calm ewes than nervous ewes but was only significant at 6 h postpartum (d.f. = 32, $t_{0.05}$, ≤1.772; Table 4). Other parameters of milk composition were not affected by selection for temperament. No interaction between time and temperament was found in any of the parameters. There were no differences in the body weight between calm and nervous lambs at birth, at 4 days or 8 days of age (Table 2).

Table 2. Lamb liveweight (mean ± s.e.) at birth, 4 days and 8 days

	Birth	4 days	8 days
Born from calm ewes	5.1 ± 0.20	6.2 ± 0.19	7.3 ± 0.30
Born from nervous ewes	5.1 ± 0.18	6.3 ± 0.23	7.8 ± 0.32

Table 3. Colostrum (mean ± s.e.) from the right mammary of single-bearing calm and nervous Merino ewes examined for quantity and viscosity at birth, 1, 3, 6 and 10 h post-partum

*$P \leq 0.05$, significant differences were observed for values between treatment groups

	Quantity (mL)		Viscosity (unit)	
	Nervous	Calm	Nervous	Calm
Birth	583 ± 97.9	441 ± 76.8	4.5 ± 1.04	4.7 ± 0.39
1 h	173 ± 19.1	150 ± 29.9	4.7 ± 1.07	5.1 ± 0.28
3 h	167 ± 41.6	110 ± 18.3	5.4 ± 1.23	5.8 ± 0.29
6 h	137 ± 16.9	134 ± 24.1	5.7 ± 1.30	6.4 ± 0.24*
10 h	148 ± 17.3	135 ± 29.8	6.3 ± 1.44	6.6 ± 0.16

There were large variations between individuals in both groups in the quality and quantity of colostrum available at birth. Assuming that both sides of the mammary produced similar quantities, 2 ewes had no colostrum available at birth. In contrast, another ewe had more than 1.7 L of colostrum available at birth. The quality also varied a great deal. The viscosity at birth ranged from being a thick, yellow-brown paste-like substance through to having properties similar to milk in regard to colour and viscosity.

Discussion

Selection for temperament does not seem to affect the quantity and composition of the colostrum of ewes. However, the colostrum of calm ewes was thinner than that of nervous ewes. Very thick or pasty colostrum would be extremely difficult, if not impossible, for a newborn lamb to suck. This view is shared by Hartmann *et al.* (1973), who considered the

Table 4. Concentration of fat, protein and lactose in colostrum produced from the right mammary of single-bearing calm and nervous Merino ewes at birth, 1, 3, 6 and 10 h post-partum

*$P \leq 0.05$, significant differences were observed for values between treatment groups

	Fat (%)		Protein (%)		Lactose (%)	
	Nervous	Calm	Nervous	Calm	Nervous	Calm
Birth	12.5 ± 2.87	10.3 ± 1.46	18.3 ± 4.20	15.7 ± 1.53	2.1 ± 0.47	2.3 ± 0.25
1 h	17.1 ± 3.91	16.6 ± 1.55	16.6 ± 3.81	14.2 ± 1.36	2.1 ± 0.48	2.3 ± 0.29
3 h	16.2 ± 3.70	15.5 ± 0.92	15.4 ± 3.53	13.3 ± 1.28	2.6 ± 0.59	2.9 ± 0.21
6 h	11.7 ± 2.68	11.3 ± 1.08	11.6 ± 2.66	10.2 ± 1.28	2.9 ± 2.67	3.5 ± 0.23*
10 h	11.7 ± 2.68*	9.3 ± 0.73	9.4 ± 2.15	7.6 ± 1.13	3.6 ± 0.83	3.8 ± 0.22

problem as being potentially life threatening to lambs. In our study there was a continuous thinning of colostrum, which seemed to increase in rate between 6 to 10 h post-partum. At this time there seemed to be a concurrent shift in the rate of change in the proportion of fat and lactose. This is consistent with reports by Murphy *et al*. (1996), Murphy (1999) and Banchero *et al*. (2004*a*, 2000*b*).

The high interindividual variability in colostrum production found in our study is consistent with the observations of Murphy *et al*. (1996), Murphy (1999), and Banchero *et al*. (2004*a*, 2000*b*). Although this could be a limiting factor to lamb survival in certain circumstances, there is little evidence to suggest that it is strongly associated with ewe temperament. Therefore, it is unlikely to explain the lower mortality reported in calm ewes. Colostrum has been shown to act as a reward to the lamb in the establishment of a unique bond with its mother as outlined by Goursaud and Nowak (1999). This would suggest that the lower mortality of lambs born to calm ewes may depend more on behavioural factors and interactions between the mother and her young rather than factors relating to colostrum.

Acknowledgments

We would like to thank Dr Roberta Bencini for technical assistance in the use of the Milkoscan 133. We also offer thanks to Mario D'Antuono for his assistance in the statistical analysis and to Steve Gray for all of his assistance and input with the on farm activities. This research was supported by Meat and Livestock Australia project AHW.085.

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Received 19 November 2005, accepted 8 April 2006

9.6 B3 Draft manuscript

Challenge by a novel and disturbing object does not impair the capacity of ewes and lambs selected for a nervous temperament to display early preference for each other

Submitted to Developmental Psychobiology

S. L. Bickell [a], R. Nowak [bcde], P. Poindron [bcde], A. Chadwick [a], D. Ferguson [f] & D. Blache[a]

[a] School of Animal Biology – M085, University of Western Australia, 35 Stirling Hwy, Crawley, Western Australia, 6009, Australia

[b] INRA, UMR85 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France

[c] CNRS, UMR6175 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France

[d] Université François Rabelais de Tours, F-37041 Tours, France

[e] Haras Nationaux, F-37380 Nouzilly, France

[f] Livestock Welfare, CSIRO Livestock Industries, Locked Bag 1, Armidale, New South Wales, 2350, Australia

Address for Correspondence:

Dr Dominique Blache

School of Animal Biology M085

Faculty of Natural & Agricultural Sciences, The University of Western Australia,

35 Stirling Highway, Crawley, WA 6009, Australia

Tel: +61 8 6488 3587

Fax: +61 8 6488 1029

Email: dbla@animals.uwa.edu.au

Running title: Temperament, novelty and ewe-lamb recognition

Abstract:
Novelty, a powerful fear inducing and stressful stimulus, could be detrimental on the capacity of ewes and lambs to recognise each other. The effect of a novel and disturbing object on the ability of ewes and lambs, selected for their calm or nervous temperament, to show a preference for each other was tested in a two-choice discrimination test. Both calm and nervous mothers spent more time near their own lamb than near the alien lamb but did not differ in any other behaviour measured. In contrast, nervous lambs showed a greater preference and took less time to reach their own mother than calm lambs. Nervous temperament does not seem to impair the capacity of ewes and lambs to display an early preference for each other and, might be an advantage in some challenging postnatal situations because of the increased motivation of nervous lambs to reunite with their mother.

Keywords: choice test, ewe-lamb recognition, novelty, sheep, temperament

Novelty is known to be a powerful fear inducing stimulus (Boissy, 1995; Désiré, Veissier, Després, & Boissy, 2004) and thus exposure to novelty can be a stressful experience for an animal. This occurs frequently in farm animals which are often exposed to novel challenges and stimuli such as new environment, new companions and unexpected stimuli (Grandin & Deesing, 1998). Such novel situations or stimuli induce levels of stress that can vary widely between individuals (Boissy, 1995; Désiré et al., 2004; Lawrence, Terlouw, & Illius, 1991) and it is generally accepted that the temperament of the animal may account for some of these differences (Gosling, 2001; Lawrence et al., 1991; Stevenson-Hinde, Stillwell-Barnes, & Zunz, 1980). For example, sheep selected for a calm temperament have a lower behavioural and cortisol stress response to a novel object than sheep selected for a nervous temperament (Bickell, 2005).

In turn, the level of stress experienced by an animal in a given situation can influence its learning and memory performances. Light stress can improve cognitive performance (McEwen & Sapolsky, 1995; Mendl, 1999; Yerkes & Dodson, 1908). In contrast, when the level of stress is too high, cognitive performances can be compromised (Mendl, 1999; Tiegen, 1994) and high levels of stress are known to impair learning and memory function in both animals and humans (De Quervain, Roozendaal, & McGaugh, 1998; Hölscher, 1999; Kim & Yoon, 1998; McEwen & Sapolsky, 1995). Therefore high levels of stress are not only detrimental to the health and wellbeing of the animal but also to the functions that rely on learning and cognition.

In sheep which naturally live in groups, specific inter-individual associations and social recognition rely on complex cognitive processes (Kendrick, 1998). This is particularly critical for the mother-young relationship, which is essential for lamb survival (Nowak & Lindsay, 1992), as there may be a conflict of interest in mothers between staying with their young and keeping contact with flock mates. Under field conditions, ewes and lambs often become separated therefore they need efficient recognition abilities and strong motivation to reunite with each other. For both members of the mother-young dyad, this recognition process relies on the memorisation of distinctive olfactory, auditory and visual characteristics (Poindron, Nowak, Lévy, Porter, & Schaal, 1993). Exposing animals to a stressful situation or stimulus, like a new environment or a novel object, could impair mother and young in their ability to make a choice or to discriminate each other by affecting attentional processes, memory retrieval, or by inducing fear responses incompatible with mother-young reunion. Several studies on pigs, sheep, cattle and deer found that agitated animals are poorer at making clear-cut choices in preference or avoidance tests (Grandin, Odde, Schutz, & Behrns, 1994; Pollard, Littlejohn, & Suttie, 1994; Rushen, 1986; van Rooijen & Metz, 1987). Stressful events around lambing are likely to increase the rate of ewe-lamb separation and to delay reunion which is in turn likely to affect the lamb's chances of survival; it could be expected that this effect is even more pronounced in nervous than in calm animals. In a study comparing two lines of sheep selected for their calm or nervous temperament, the percentage of lambs dying between birth and weaning in the calm temperament line was half of that of the nervous one, an observation made in both single- and twin-born lambs (Murphy, Lindsay, & Le Neindre, 1998). Results from our previous study have shown that ewes and lambs, whether calm or nervous, show little difference in their ability to discriminate each other in a two-choice test (Bickell, Nowak, Poindron, Sebe, Chadwick, Ferguson, & Blache, 2009), demonstrating that in mother and young the initiation of the bonding process is not affected by temperament. However, this experiment was run indoors under optimal conditions, which avoided any source of stress. This does not exclude that in a much more challenging situation, the recognition performances of nervous sheep would be lower than those of calm sheep. As sheep that have been selected for their nervous reactivity are known to be more stressed by novelty than calm sheep (Beausoleil, Blache, Stafford, Mellor, & Noble, 2008; Nowak, Poindron, Sèbe, Hart, Chadwick, & Blache, 2006), then exposure to a novel object may impair the mother-young discriminative ability in nervous sheep more than in calm sheep.

This study investigated to what extent novelty would affect the recognition abilities of calm and nervous ewes and lambs using a two-choice test. It was hypothesised that, tested in a new environment and in the presence of a novel and disturbing object, ewes and lambs selected for their calm temperament would both be better at recognising their own kin than ewes and lambs selected for a nervous temperament. Based on previous studies (Keller, 2003; Nowak, 1990; Terrazas, Ferreira, Levy, Nowak, Serafin, Orgeur, Soto, & Poindron, 1999), the ability of ewes and lambs to show a preference for each other was tested at 6 hours after parturition in mothers and 18 hours after birth in lambs to detect potential differences between the two temperament lines in this challenging situation.

MATERIALS AND METHODS

Animals and housing conditions

Thirty pregnant Merino ewes (15 calm, 15 nervous) were selected from the temperament flock maintained at the Allandale Research Farm of the UWA School of Animal Biology, Wundowie, 70 km east of Perth. For selection purposes, the temperament had been tested in individual lambs when they were three months old, two weeks after weaning, using the two behavioural tests described by Murphy et al. (1994). In brief, the first behavioural test is a conflict of motivation test between approaching flock members and a human in an arena, the second test is an isolation test in a closed box (1.5 m^3). A score based on movements and vocalisation, combining the behaviour of the subject in the two tests, is given to each individual and used for selection, highest scores corresponding to most nervous subjects.
For this experiment, all ewes had been synchronised and artificially inseminated on a single day by semen from a sire of the same temperament line (calm or nervous). Each ewe had previously lambed in earlier years (multiparous) and females were all determined to be carrying a single lamb by ultrasound scanning. Three weeks before parturition, ewes were transferred indoors to individual deep litter pens measuring 1.2 x 3.2 m. They were fed hay and concentrates according to the physiological requirements of their stage of pregnancy and had water available *ad libitum*. The use of animals and the procedure were approved by the Animal Ethics Committee of the University of Western Australia (approval number 05100466).

Experimental procedures

Recognition of the lamb by mothers 6 h after parturition

The testing arena and procedure were similar to those described by Bickell et al. (2009). A triangular testing arena was built in an isolated room situated 50 m away from the group of lambing ewes (Figure 1). The base of the triangle consisted of three pens side by side. The two corner pens contained a stimulus lamb (own or alien) and were separated by a pen containing a novel object, 100 cm in height. The object was powered by an electric fan and blew air into a white soft plastic tube (20cm in diameter, 50cm in length) so that the tube moved at random and made moderate noise. The object was mobile, noisy and novel, characteristics that are psychologically stressful for animals (Boissy, 1995; Désiré et al., 2004) and had also been demonstrated to be a fear inducing stimulus (Bickell 2005). These three pens were separated from the testing area by a set of hurdles, 1 m away, so that the ewes could see and hear the lambs but not smell them. This testing pen was designed

from previous studies (Ferreira, Terrazas, Poindron, Nowak, Orgeur, & Levy, 2000; Terrazas et al., 1999) showing that under such conditions, recognition of the lambs is more challenging. The testing arena was divided into five zones and only an area of 1x2 m in front of each lamb was considered as a zone of proximal contact between the ewe being tested and the stimulus lambs.
The test was conducted 6 hours after parturition since olfactory recognition of the young by the ewe requires only 1/2 to two hours of contact (Keller, 2003) and there is evidence that recognition without the help of olfactory cues is established within 6 to 8 h post-partum in multiparous mothers (Keller, 2003; Terrazas et al., 1999). In each genetic line, half of the mothers were tested with their own lamb on either the right or the left side of the arena. The test lasted for 5 minutes and started once the novel object was switched on and the ewe was released. The test was videotaped so that it could be analysed later in the laboratory (Table 1); however, vocal activity was noted during the test by at least two experimenters, as the source of each bleat could not be identified on the video-recordings.

Preference for the mother by 18-h-old lambs

Although there is no doubt that lambs can recognise their mother once they are two days old (Nowak, 1990; Sèbe, Nowak, Poindron, & Aubin, 2007), there is evidence that in the first day the orientation response depends mainly on the display of maternal acceptance by the own mother (Terrazas, Nowak, Serafin, Ferreira, Lévy, & Poindron, 2002). Therefore the term preference was chosen instead of recognition in the study concerning lambs.

The expression of a preference for the mother was assessed in a two-choice test when lambs were 18-h old according to the design of the testing arena and protocol described by Bickell et al. (2009). The testing arena was derived from the one used in the recognition of the lamb by the mother (Figure 1). However, the zone of contact was reduced to be a 50 cm-wide area in front of the stimulus ewes.

In each line of sheep, half of the lambs were tested with their mother on either the right or the left side of the arena. The test lasted for 5 minutes and the procedure, data recording (Table 1) and data analysis was the same as for mothers

Statistical analyses

To detect differences in the ratio of calm and nervous animals that reached the proximity or contact zone a Fisher's Exact probability test was performed. To detect differences between temperament lines a Mann-Whitney test was used and a Wilcoxon test for paired samples was used to detect differences between own versus alien. Due to incomplete data recording because of technical problems with the recording equipment, 1 calm ewe had to be excluded from the study; however, this does not apply for lambs, which were all tested in both groups.

RESULTS

Recognition of lambs by their mothers 6 h after parturition

All the nervous ewes and all but one calm ewe reached the proximity zone in front of any lamb and their own lamb before the end of the test. Mothers from both temperament lines did not differ in the time it took for them at the start of the test to initially reach any proximity zone (Calm = 26.3 ± 21 sec; Nervous = 3.2 ± 0.7 sec, p = 0.15) or to reach the proximity zone in front of their own lamb (Calm = 29 ± 21 sec; Nervous = 8.3 ± 2.1 sec, p = 0.93).

The time calm and nervous ewes spent next to or looking at each lamb did not differ between temperament lines and both calm and nervous mothers spent more time near their own lamb than near the alien lamb (Calm p = 0.01; Nervous p = 0.03, Figure 2). Nervous ewes looked at their own lamb more than at the alien lamb (p = 0.03), while no differences were detected in the amount of time calm ewes spent looking at each lamb (Figure 2).

The number of visits made to each proximity zone did not differ between calm and nervous mothers (Own lamb: Calm = 6 ± 0.8; Nervous = 9.4 ± 1.6, p = 0.19, Alien lamb: Calm = 4.8 ± 0.8; Nervous = 7.7 ± 1.2, p = 0.08), and mothers of both temperament lines visited more the zone located next to their own lamb than to alien lamb (Calm p = 0.02; Nervous p = 0.03). The number of visits to each proximity zone tended to differ between temperament lines, as the nervous ewes tended to cross these zones more than the calm ewes (Nervous = 17 ± 2.7; Calm = 10.8 ± 1.5, p = 0.059).

Vocal activity also differed between the two temperament lines, with nervous mothers emitting more high-pitched bleats than calm mothers during the test (Nervous = 103 ± 6.2; Calm = 80 ± 6.9, p = 0.011). Nonetheless, calm ewes emitted more high pitched bleats near their own lamb than near the alien lamb (Own lamb = 34.7 ± 5, Alien lamb = 18.9 ± 3.7, p = 0.02), while this difference did not quite reach significance for nervous ewes (Own lamb = 41.2 ± 4.3, Alien lamb = 29.1 ± 4.4, p = 0.08). Finally, the vocal activity of the stimulus lambs did not differ between temperament lines, nor between the own and the alien lamb (Calm own = 43.2 ± 7; Nervous own = 49.6 ± 10, Calm alien = 49.8 ± 9.2; Nervous alien = 49.7 ± 8.6).

Preference for the mother by 18-h-old lambs

Two calm and one nervous lamb did not reach any contact zone before the test finished (Fisher exact p = 0.61), while six calm and two nervous lambs failed to reach the contact zone in front of their own mother before the test finished (Fisher exact p = 0.22). Calm lambs took longer than nervous lambs to reach the zone of contact in front of any ewe (Calm = 130 ± 30 sec; Nervous = 48 ± 19 sec, p = 0.015) and to reach the contact zone in front of their own mother (Calm = 183 ± 28 sec; Nervous = 91 ± 25 sec, p = 0.01).

The time that lambs spent near or looking at their mother differed between the two temperament lines (Figure 3). Nervous lambs spent more time near their own mother (p = 0.008), and looked at their own mother more than at the alien ewe (p = 0.004), while the time calm lambs spent near (p = 0.221) or looking (p = 0.347) at each ewe did not differ significantly (Figure 3). Also, the time spent near (p = 0.375) or looking (p = 0.205) at the alien ewe did not differ between the lambs of the two temperament lines (Figure 3).

The number of visits made to each ewe did not differ between temperament lines (Own ewe: Calm = 0.8 ± 0.2; Nervous = 1.3 ± 0.2, p = 0.08, Alien ewe: Calm = 0.9 ± 0.3; Nervous = 1.1 ± 0.2, p = 0.19) and the number of times lambs changed contact zones did not differ either between calm and nervous lambs (Calm = 1.7 ± 0.4; Nervous = 2.5 ± 0.4, p = 0.21).

The number of high-pitched bleats the lambs emitted in each proximity zone near each ewe did not differ between the two temperament lines (Calm: Own ewe = 1.3 ± 0.6, Alien ewe = 2.6 ± 1, p = 0.44; Nervous: Own ewe = 9.4 ± 2.9, Alien ewe = 17 ± 6.1, p = 0.3) nor did the total number of bleats emitted by the lambs (Calm = 34 ± 4.3; Nervous = 44.5 ± 8.4, p = 0.62). Finally, the vocal activity of the stimulus ewes did not differ between temperament lines, or between the own and the alien ewe (Calm own = 74 ± 5; Nervous own = 72.4 ± 6.8, Calm alien = 70 ± 5.7; Nervous alien = 80.3 ± 11.4).

DISCUSSION

The hypothesis that in the presence of a novel object a nervous temperament would impair the ability of ewes and lambs to identify each other and to reunite in a two-choice test was not supported by our results. In fact, the opposite was obtained in lambs since nervous animals expressed better discriminative abilities, in terms of time spent near the dam, or time spent looking at her, than calm lambs. A greater level of motivation to reach the proximity of their mothers in the nervous lambs than in the calm lambs could explain the lack of preference in the calm lambs. It is possible however, that calm and nervous lambs adopted different coping mechanisms when they were exposed to a challenging situation. Different coping mechanisms was also possibly observed in mothers as only the nervous ewes spent significantly more time looking at their own lamb. Although the difference was not as pronounced as in lambs, it is consistent with our previous results in favour of a better expression of maternal recognition in the nervous line (Bickell et al., 2009).

One explanation for the better discriminative abilities of nervous lambs may lie in a stronger motivation to seek the proximity of their mother compared with the calm lambs. Fear heightens an individual's motivation to cope effectively with an impending stressor (Baron, Logan, Lilly, Inman, & Brennan, 1994) and thus the nervous lambs may have perceived the novel object as something fearful, increasing their motivation to reunite with their mothers rather than avoiding the object as we thought. The object was mobile, noisy and novel, characteristics that are psychologically stressful for animals (Boissy, 1995; Désiré et al., 2004). The nervous lambs, because of their predisposed nervous temperament, may have perceived the novel object as something negative and stressful which increased their motivation to seek comfort and therefore to reunite with their mothers. Indeed, nervous lambs were quicker than calm lambs to reach their mothers and thus performed better in the recognition test. By contrast, the calm lambs, because the novel object was less frightful to them, took more time to reach their mother and were not as motivated to stay next to her. The fact that the vocal activity of calm lambs, once near their mother, is less important than in nervous lambs is in favour of this argument.

Alternatively, exposure to the novel object may have been just as fearful to the calm lambs as it was to the nervous lambs. We have previously shown that calm and nervous lambs do not differ in their performance when tested in the very same conditions but in the absence of the novel object, both temperament lines being able to show a clear preference for their mother (Bickell et al., 2009). Therefore, the difference found in the present study between calm and nervous lambs is due to their reactivity to the novel object, not the novel environment. Unexpectedly, the addition of the novel object to the two-choice situation changed the response of the calm lambs but not that of the nervous lambs. One explanation for this unexpected outcome is that the two-choice situation might have been already highly stressful to the nervous lambs and thus any additional stressor made no difference to their motivation to reunite with their mother. In contrast, in the calm lambs, the addition of the stressor increased their level of stress, or induced stress, that resulted in adopting a withdrawal response to the stressful item. In fact, active seeking of the ewe by the nervous lambs and avoidance behaviour of the calm lambs seem to be a characteristic of their temperament. When tested at weaning in an arena test evaluating conflicting motivations, social attractiveness towards flock mates and avoidance of a human, the nervous lambs approach the human standing in front of the flock-mates while calm animals will avoid the human (Beausoleil et al., 2008). Although the human is perceived as a stressful stimulus by sheep reared under extensive conditions, the need to stay close to flock-mates in nervous animals may override their usual fear of humans while the reverse is observed in calm animals which prefer to keep away from the human.

The difference in the lamb's performance could therefore be because calm and nervous lambs adopt different coping strategies when they are exposed to a challenging situation.

When animals are faced with stressful situations they adopt behavioural strategies to try and cope with them (Koolhaas, Korte, De Boer, Van Der Vegt, Van Reenen, Hopster, De Jong, Ruis, & Blokhuis, 1999). Calm lambs would predictably react to the novel object with avoidance behaviour, somewhat equivalent to the conservation-withdrawal response (Koolhaas et al., 1999). The novel object was positioned between the ewes and thus to approach their mother the lambs had to approach the novel object as well. Calm lambs took longer to approach the contact zone than nervous lambs. This suggests that calm lambs were avoiding the novel object rather than a lack of recognition of the mother since a previous study demonstrated that preference were similar between lamb of both temperament lines (Bickell et al., 2009). On the other hand, nervous lambs may have adopted a more active style of coping as suggested by (Beausoleil et al., 2008). Nervous lambs might have actively sought the proximity of their mother because their mother represented safety (Lansade, Bouissou, & Boivin, 2007).

Unlike the lambs, the calm and nervous ewes did not differ much in their recognition abilities indicating that their motivation to reach their lambs overrode their fear of novelty. Only the time spent looking at either their own or the alien lamb differed between the two lines. The ewes were both as able to recognise their own lambs from an alien lamb even in the presence of the novel stressor. This may suggest that the adult animals are not as stressed by the novel object as neonates could be. However, previous research in a completely different situation has found that the object used in the present study is indeed a source of stress, especially for the nervous ewes, (Bickell, 2005). The high frequency of high-pitched bleats and movements in the testing arena indicates that the ewes were in fact stressed, either by the novel object or the situation or both. The two-choice situation itself is quite challenging for the ewes as they cannot escape out of the pen nor can they make proper contact with their lambs. It cannot be excluded that the addition of the novel object may have had little effect on increasing the challenge or stress of the situation. Despite this stressful situation, the ewes did recognise their young and this suggests that the motivation of mothers to reach the proximity zone in front of their lambs overrode their fear of novelty. Many studies have found a reduction in fearfulness in pregnant and lactating females (Hard & Hansen, 1985; Maiestripieri & D'Amato, 1991; Viérin & Bouissou, 2007) as this reduction in fearfulness serves to facilitate the expression of defensive aggression in protection of the young (Turner & Lawrence, 2007). In the current study, there was the increased motivation to reunite with the attachment partner due to the fear inducing novel object which was not present in the previous research (Bickell et al., 2009).

Our study has shown that the selection for a nervous temperament does not impair the capacity of ewes and lambs to display an early preference for each other when exposed to a novel and disturbing object and tested in a two-choice testing situation. In fact, having a nervous temperament may prove to be an advantage in some challenging fear inducing situations since it seems to increase the motivation of nervous lambs to reunite with their mother. However, this work was conducted in an experimental and spatially confined testing situation. Whether having a nervous temperament would be an advantage in a natural outdoor context where animals can express flight responses needs further investigation. Nonetheless our study highlights that the higher lamb mortality found under experimental field conditions in the nervous line by Murphy et al. (1998) is unlikely to be due to any social cognitive deficiency.

Notes

We would like to thank Céline Lenoury, Ken Hart, Baptiste Deleplace and Monique Poindron for all their help with the experimental work. We would also like to thank Steve Gray for all his help, advice and expertise and for caring for the animals. The work was supported by funding and a PhD scholarship from Meat and Livestock Australia and The University of Western Australia.

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Table 1: Variables recorded during the 5-minute two-choice discrimination test.

Locomotor activity of tested animal
Latency to reach a proximity / contact zone; reach being defined as the head and front legs in the proximity / contact zone
Total time spent in each proximity / contact zone; animals were considered in the proximity / contact zone if their head and front legs were in the zone
Total number of visits to each proximity / contact zone
Time spent looking (Terrazas et al., 1999) at each stimulus animal
Vocal activity of tested animal and stimulus animals
Number of high-pitched bleats (open mouth bleat)
Number of low-pitched bleats (closed mouth bleat)

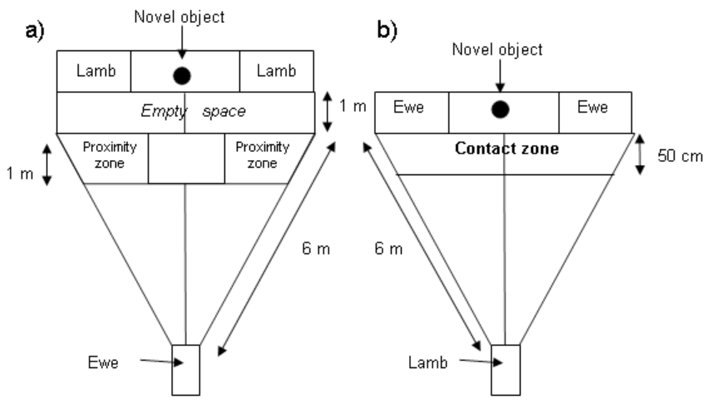

Figure 1: Testing pens used in the recognition of the young by the ewe (a) and in the preference for the mother by the lamb (b).

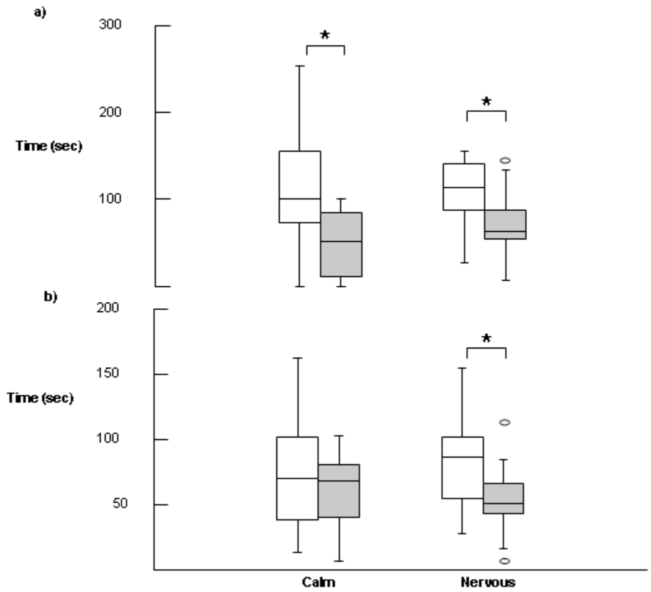

Figure 2: Time spent by calm (n = 14) and nervous (n = 15) ewes near (a) or looking at (b) their own (white boxes) or alien (grey boxes) lamb during the two choice test conduced at 6 hours after parturition. Values are medians (bar within the box) and first and third quartile ranges (boarders of box) with 10 and 90 percentile shown as the error bars. Outliers are shown as circles. *: $p < 0.05$.

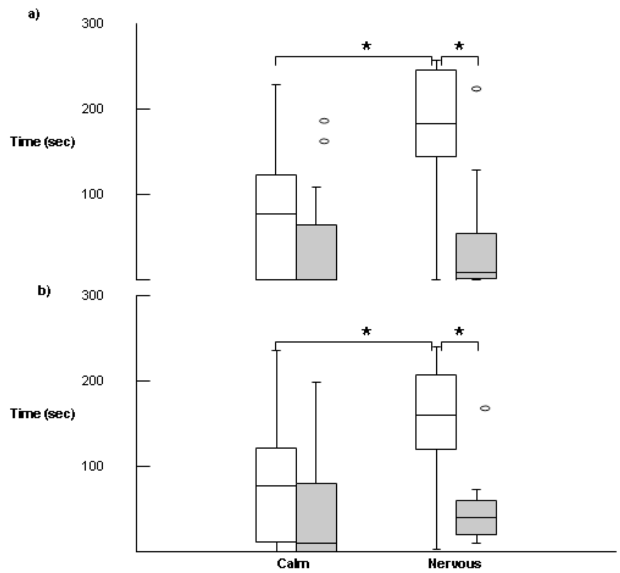

Figure 3: Time spent by calm (n = 15) and nervous (n = 15) lambs near (a) or looking at (b) their own (white boxes) or alien (grey boxes) ewe during the two choice test conducted at 18 hours after birth. Values are medians (bar within the box) and first and third quartile ranges (boarders of box) with 10 and 90 percentile shown as the error bars. Outliers are shown as circles. *: $p < 0.05$.

10 Acknowledgements

We would like to especially acknowledge the excellent co-operation and support from of the eight industry collaborators. This project would not have been possible without their collaboration. These include Bill Sandilands (Billandri), Roland Ritson (Grindon) Ian Roberston (Merinotech), Nick and Janelle Hocking-Edwards (Washpool), Graig Heggaton (Genstock), Max Watts (Gnaring Park) Greg Sargeant (Eagleridge) and James Skerritt (Cathair Donal).

We would also like to acknowledge the technical skill and professionalism of our research teams at UWA and CLI in particular, Aprille Chadwick, Jim Lea and Dom Niemeyer.