

# The welfare status of newborn lambs

## Newborn lamb welfare

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# 1. EEG responses of lightly anaesthetised newborn and young lambs to an electrical stimulus: The effect of pregnanolone and GABA<sub>A</sub> receptor antagonist picrotoxin

## 1.1. GOAL

To assess the impact on newborn lamb welfare of naturally produced suppressors of awareness with anaesthetic, sedative and analgesic properties during the first week following birth.

## 1.2. CONTEXT OF THE PROJECT

New Zealand needs to prepare a measured and rational response to international criticism of its average 20 percent annual lamb loss rate. In addition to continuing research into the causes and prevention of neonatal loss, research needs to be undertaken to assess the extent of suffering that newborn lambs may experience prior to death and during morbidity. Previous work has shown that cerebral responses to castration in anaesthetised lambs are reduced for the first few days after birth (Johnson *et al.*, 2009). This suggests that during this time lambs may experience less pain when exposed to adverse conditions and that the extent of suffering may therefore be reduced. The present project investigated the potential for residual suppressors of awareness to dull pain perception in lambs over the first week after birth by assessing EEG responsiveness to infusion of the suppressor pregnanolone and an antagonist acting via the same receptors.

## 1.3. APPROACH

Healthy twin lambs 4 to 24 hours (newborn lambs, n=37) and 7 to 11 days (young lambs, n=38) of age were anaesthetised and endtidal halothane concentrations were titrated to 1.0 percent. The electroencephalogram (EEG) and electrocardiogram (ECG) were recorded continuously at a sampling rate of 1kHz and analysed offline at the end of the experiment.

Lambs of each age group were assigned to three infusion groups. These included a control group (infusion of 1ml Intralipid®), a 'suppressor of awareness' group (infusion of 5mg pregnanolone in 1ml Intralipid®) and a 'GABA<sub>A</sub> receptor antagonist' group (infusion of 0.1mg/kg picrotoxin in 1ml saline). For all lambs 15 minutes of baseline EEG and ECG were recorded. Thereafter, lambs received a 1ml-infusion over five minutes. Once infusion was completed, lambs received three electrical stimuli (2 seconds, 75V, 50Hz) which were each followed by a 10-minute recording period. An exception were control lambs, which had the first electrical stimulus presented before the infusion began. The ECGs before and after each stimulus were analysed for heart rate (BPM). The median frequency (F50), spectral edge frequency (F95) and total power (P<sub>tot</sub>) for consecutive 1-second segments as well as the power for frequencies of 1 to 30 Hz were calculated from EEGs before and after each stimulus. The relative contribution to total power (relative power) of the frequency ranges of 1 to 4 Hz, 5 to 8Hz, 9 to 12 Hz and 13 to 30 Hz was determined.

The experimental protocol was approved by the Massey University Animal Ethics Committee (Protocol # 05/65).

## 1.4. OUTCOMES

Overall, the results of the present study were inconclusive. Although the overall responsiveness of newborn lambs seemed to be less than that of young lambs judged by the number of parameters in which significant changes were observed, the responsiveness of infusion lambs to the electrical stimuli was not what would have been expected if these factors had been involved in suppression of awareness or analgesia. Both newborn and young lambs showed significant changes in a variety of parameters in response to the stimuli after pregnanolone infusion. In addition, it was anticipated that the receptor antagonist picrotoxin would increase the response to stimulation, antagonising the actions of any residual suppressors of awareness present. In fact, this did occur to a small extent in newborn lambs, whereas the responsiveness in young lambs was similar to that of pregnanolone infusion lambs.

The complexity of the study and several limitations in experimental design which only became evident after completion of the study may have been responsible for the inconclusive data. First, despite the fact that there was a control group at each age studied, it cannot be ascertained whether the EEG responses in lambs receiving an infusion were due to the infusion *per se* or to electrical stimulation. Second, it is possible that the electrical stimuli presented (3 x 2 seconds) were not strong enough or not presented for a sufficiently long period to elicit a consistent response. Previous studies that were able to detect significant responses in the EEG of anaesthetised animals to noxious stimulation used somato-visceral stimulation (e.g. castration) lasting at least 90 minutes rather than short-lived electrical stimuli. Also, EEG responsiveness may be different depending on the modalities involved, i.e. predominantly systemic cutaneous receptors for castration and mainly visceral for ring castration. Third, picrotoxin is not a direct antagonist of suppressors of awareness, although it acts via the same receptor. Therefore, any effect observed may have been unrelated to the presence of suppressors of awareness. Lastly, it is possible that the concentrations of pregnanolone and picrotoxin were not sufficient to bring about the anticipated effects.

Although inconclusive in these respects, consideration of the reasons for this led to improved experimental designs and more informed interpretation of subsequent studies of lambs and comparisons with rat pups and wallaby joeys (not funded by MAF) (Diesch et al., 2009, 2010).

## 1.5. SUMMARY

The present project investigated the potential of residual suppressors of awareness to dull pain perception in lambs over the first week after birth by assessing EEG responsiveness to noxious stimulation in lightly anaesthetised lambs.

Newborn (4 to 24 hrs) and young lambs (7 to 11 days) were anaesthetised and maintained on a light plane of anaesthesia. EEG and ECG were recorded continuously throughout the study. Lambs of each age received one of three infusions; vehicle control, suppressor of awareness or receptor antagonist. Three electrical stimuli were presented to the lambs followed by a 10-minute recording period. The EEGs and ECGs before each stimulus were compared to those thereafter.

The results of the study were inconclusive. Although newborn lambs overall responded less to the stimuli than young lambs, the effects of the suppressor of awareness and the receptor antagonist *at the dose rates used* were not indicative of any analgesic effects or suppression of awareness (suppressor) or the removal of such (antagonist). This could be due to the complexity of the study and several limitations in experimental design which only became evident after completion of the study.

## 1.6. PUBLICATIONS

- Diesch TJ, Mellor DJ, Johnson CB & Lentle RG (2009). Electroencephalographic responses to tail clamping in anaesthetised rat pups. *Laboratory Animals* **43**, 224-231.
- Diesch TJ, Mellor DJ, Johnson CB & Lentle RG (2010). Developmental changes in the electroencephalogram and responses to a noxious stimulus in anaesthetized tammar wallaby joeys (*Macropus eugenii eugenii*). *Laboratory Animals* (**44**, 79-87).

## 1.7. REFERENCES

- Johnson CB, Sylvester SP, Stafford KJ, Mitchinson SL, Ward RN & Mellor DJ (2009). Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen from birth to six weeks old. *Veterinary Anaesthesia and Analgesia* **36**, 273-279.

## 1.8. NOTE

Information on the two related projects carried out concurrently to this study will be provided in form of an appendix. Copies of any publications relating to these projects will also be forwarded once available. In addition, information collected in two separate studies on wallaby joeys and rat pups, pertaining to development of conscious perception, are relevant to newborn lambs and this has been published in the reviews by Diesch *et al.* (2008) and Mellor *et al.* (2009, 2010) listed below.

- Diesch TJ, Mellor DJ, Johnson CB & Lentle RG. (2008). Responsiveness to painful stimuli in anaesthetised newborn and young animals of varying neurological maturity (lambs, rat pups and wallaby joeys). *Alternatives to Animal Testing and Experimentation* **14**, 549-552.
- Mellor DJ, Diesch TJ & Johnson CB. (2009). Legal and animal welfare implications of when consciousness first appears in developing young and of the potential for delayed onset of increased pain sensitivity. In: *The Welfare of Animals – It's everyone's business*. Proceedings of the Australian Animal Welfare Strategy International Conference, Conrad Jupiters, Gold Coast, Queensland, Australia, 31 August to 3 September 2008: [http://www.daff.gov.au/\\_data/assets/pdf\\_file/0019/1046431/25-craig-johnson.pdf](http://www.daff.gov.au/_data/assets/pdf_file/0019/1046431/25-craig-johnson.pdf) (accessed 3 April 2009).
- Mellor DJ, Diesch TJ & Johnson CB. (2010). Should mammalian fetuses be excluded from regulations protecting animals during experiments? *Alternatives to Animal Testing and Experimentation – Proceedings of the 7<sup>th</sup> World Congress on Alternatives and Animal Use in the Life Sciences*, **Special Issue**, 199-202.
- Mellor DJ, Diesch TJ & Johnson CB. (2010). When do mammalian young become sentient? *Alternatives to Animal Testing and Experimentation – Proceedings of the 7<sup>th</sup> World Congress on Alternatives and Animal Use in the Life Science*, **Special Issue**, 275-280.

## 2. Appendix 1: Ontogeny of the electroencephalogram and associated behaviours of lambs over the first 2 days after birth

### 2.1. GOAL

To determine whether changes occur in the EEG of an animal species born neurologically mature over the first few minutes and days after birth. Such changes, if present, may indicate neurological development beyond birth associated with exposure to a new environment or the potential for modulation of consciousness perception.

### 2.2. CONTEXT OF THE PROJECT

Sheep are a precocial species, in which by definition most neurological development occurs before birth. However, conscious perception appears to be precluded prenatally due to the operation *in utero* of a range of potent neuroinhibitory mechanisms that maintain states of unconsciousness in the mature fetus throughout the last half of pregnancy (Mellor *et al.*, 2005; Mellor & Diesch, 2006). Hence, the evidence suggests that conscious perception usually does not occur until some time after birth. However, when after birth consciousness, and thus conscious perception, may occur for the first time is, as far as can be ascertained, not known. Behavioural arousal is observed in the normal healthy newborn very soon after the onset of rhythmic breathing. Its features include behaviour that is suggestive of conscious perception, such as interactions with the dam and, once the lamb is able to stand successfully, seeking the teat. However, it is possible that full consciousness is *not* necessary for such behaviours to occur. Increased arousal may not necessarily include conscious perception, as it is possible to be awake but unconscious (Mellor *et al.*, 2005).

### 2.3. APPROACH

Romney ewes close to birth were calmly herded into a semi-enclosed barn, where they were kept for several hours after giving birth to ensure bonding with their lambs.

As soon as the lambs were free of the birth canal electrodes for electroencephalographic (EEG) recordings were placed and EEG recording commenced immediately. A video camera was used to record the behaviour of the lambs. The EEG and behaviour were recorded for up to 30 minutes after birth. After the initial recordings were concluded, rectal temperature was measured, the lamb was weighed and a blood sample was taken by jugular venepuncture. Lambs were returned to their dam and bonding was ensured. Once the lambs suckled from the udder and had become sleepy another 5 to 15 minutes of EEG were recorded (i.e. between 1 and 4 hours after birth). In five lambs, ten minutes of EEG were recorded at about one day (n=2) or about two days (n=3) after birth.

EEGs were assessed visually and assigned to one of three states, low voltage high frequency EEG (LVHF), high voltage low frequency EEG (HVLF) or intermediate EEG (INT). Data assessed included the median frequency (F50), spectral edge frequency (F95) and total power (Ptot), as well as the individual frequency ranges of the EEGs. Each of these parameters has interpretative significance.

This research was approved by the Massey University Animal Ethics Committee (Protocol 06/40).

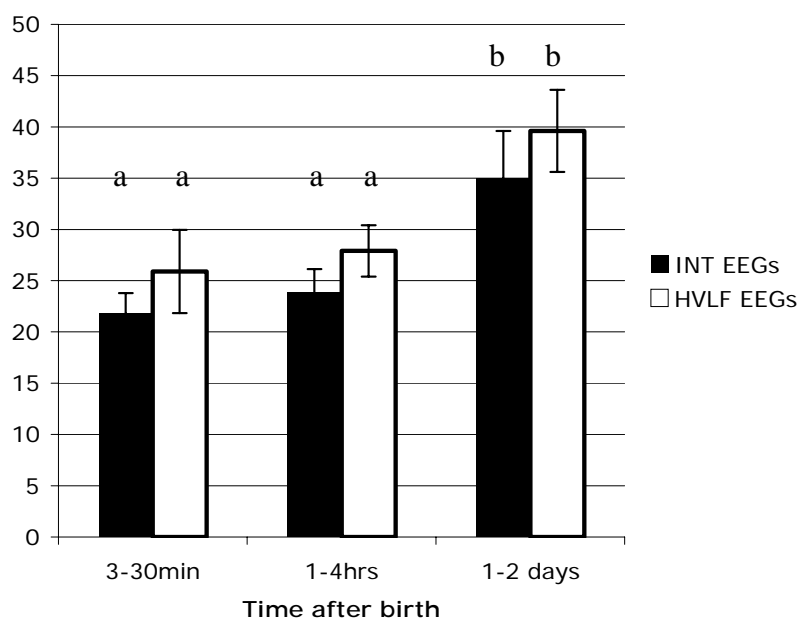
## 2.4. OUTCOMES

Overall there were no differences in any EEG parameters between LVHF and INT EEGs and hence comparisons focussed on INT and HVLF EEGs, as LVHF EEGs were absent at 1-2 days. In lambs 1-4hrs and 1-2 days after birth, F50 was significantly higher in INT EEGs than HVLF EEGs ( $p=0.049$  and  $p=0.039$ , respectively). In addition, significant differences in EEG power between INT and HVLF EEGs were observed for 1-4hr-old lambs in the frequency ranges of 1-4Hz, 4-6Hz, 10-12Hz, 13-15Hz and 19-21Hz, and for 1-2-day-old lambs in the frequency bands of 1-3Hz, 16-18Hz and 19-21Hz.

There was a decrease in the presence of movement artefacts in EEG traces with time after birth. The proportion of LVHF EEGs decreased with age, such that LVHF EEG was not observed in lambs 1 to 2 days after birth (Table 1). In contrast, HVLF EEG was absent between 3 and 15 minutes after birth, its incidence low between 15 and 30 minutes after birth and the proportion of HVLF EEG increased with time after birth (Table 1). When comparing all age groups, P<sub>tot</sub> increased with time after birth in INT ( $p=0.027$ ) and HVLF ( $p=0.023$ ) EEGs (Figure 1). In addition, relative EEG power of the frequency bands of 16-18Hz, 22-24Hz, 25-27Hz and 28-30Hz decreased with time after birth in INT EEGs.

**Table 1: Percentage contribution of the three EEG states to EEG data in the three age groups (with ^ and without + consideration of movement artefact).**

Time after birth	LVHF	INT	HVLF	Artefact
3 to 30 minutes	45.9%+ 6.7%^	48.7% 7.1%	5.4% 0.8%	85.4%
1 to 4 hours	12.2%+ 6.6%^	30.6% 16.7%	57.2% 31.1%	45.6%
1 to 2 days	0%+ 0%^	34.4% 26.0%	65.6% 49.6%	24.4%



**Figure 1: Total power for INT and HVLF EEGs of lambs of the three age groups. Values not sharing the same letter are significantly different from each other,  $p<0.05$ .**

With regard to behaviour, there was a significant decrease in the number of whole body movements ( $p=0.007$ ) and sucking attempts ( $p=0.002$ ) with time after birth. As would be expected, behaviours associated with the onset of breathing (i.e. head shake and sneeze) decreased significantly between 3 to 15 minutes and 1 to 4 hours after birth ( $p=0.002$  and  $p<0.001$ , respectively).

## 2.5. SUMMARY

While lambs are evidently in a state of heightened arousal soon after birth, this is not necessarily indicative of conscious perception, as arousal can be mediated by lower brain centres without involving consciousness. Thus, it cannot be ascertained from the results of the present study whether the arousal observed in the present lambs was associated with conscious perception. Further studies would be necessary to elucidate this.

The onset of conscious perception in newborn lambs would not be expected to be immediate, like switching on a light; instead it may be a more gradual process lasting at least several minutes and possibly up to several hours. Thus, conscious perception may initially be qualitatively different from that of young and adult sheep. However, this may not preclude animals at this stage from suffering in response to noxious stimulation and they should be given the benefit of doubt when invasive procedures are being considered.

## 2.6. PUBLICATIONS

Manuscript in preparation.

## 2.7. REFERENCES

- Mellor DJ & Diesch TJ. (2006). Onset of sentience: The potential for suffering in fetal and newborn farm animals. *Applied Animal Behaviour Science* **100**, 48-57.
- Mellor DJ, Diesch TJ, Gunn AJ & Bennet L. (2005). The importance of 'awareness' for understanding fetal pain. *Brain Research Reviews* **49**, 455-471.

### 3. Appendix 2: Plasma and brain allopregnanolone concentrations in newborn lambs between birth and 9 days of age

#### 3.1. GOAL

To measure the concentrations of the neuroactive steroid allopregnanolone over the first 9 days after birth in plasma and/or brain tissue of newborn lambs to evaluate its potential for modulation of conscious perception in animals born neurologically mature.

#### 3.2. CONTEXT OF THE PROJECT

Evidence suggests that conscious perception might initially be different in neonatal lambs compared to older animals despite their relative neurological maturity at birth. First, changes in the composition of electroencephalographic (EEG) spectra seem to occur during the first 1 to 2 days after birth (see Study 1 above). In addition, a recent study by Johnson *et al.* (2009) showed that there were significant changes in responsiveness of the cerebral cortex to castration in lambs between birth and 5 weeks of age, especially over the first 10 days, during which the response increased markedly from very low levels during the first 1 to 3 days after birth.

Neuroinhibitory agents involved in maintaining unconscious sleep-like states prior to birth, such as the potent neuroactive steroid allopregnanolone, may still be present in sufficiently high concentrations during the first few days after birth to exert their neuroinhibitory effects despite the presence, initially, of activating substances (Mellor & Diesch, 2006).

#### 3.3. APPROACH

Lambs (Romney/Finn/Texel X) were aged between 4 and 12hrs, ~24hrs, ~36hrs, ~72hrs, ~7 days and ~9 days after birth (n=5 per group) and were euthanased by overdose of pentobarbitone sodium administered following removal a blood sample via jugular venepuncture. Brain samples were maintained frozen on dry ice and included the spinal cord (base of skull), medulla, pons, cerebellum, basal ganglia, hippocampus and cerebral cortex. Samples were frozen at -80°C at the end of the day and subsequently maintained at -30°C until further analysed. The study was approved by the Massey University Animal Ethics Committee (Protocol No. 04/93).

Allopregnanolone was extracted from brain tissue and plasma by modification of a method used by Barbaccia *et al.* (1992), as described by Yawno *et al.* (2007). Allopregnanolone concentrations in brain tissue and plasma were measured in duplicate by a previously validated specific radioimmunoassay (RIA).

Protein concentrations of brain tissue samples were assessed by the Lowry protein assay to determine the concentrations of allopregnanolone per mg of protein rather than per unit wet weight thus taking into consideration any changes in tissue water content with age.

### 3.4. OUTCOMES

Plasma allopregnanolone concentrations of lambs aged up to 12hrs were significantly higher than those of older lambs ( $p=0.043$ ; Figure 2). Overall, there was a gradual decline in the concentrations with age, with values from 1.5 days showing little subsequent change.

Changes in brain tissue allopregnanolone concentrations were only observed in the medulla ( $p=0.046$ ). However, once significant changes in protein concentrations with age ( $p=0.006$ ) were taken into consideration, there were no overall changes in the medullar allopregnanolone concentrations ( $p=0.122$ ). Age-related concentration changes in other brain areas were not significant.

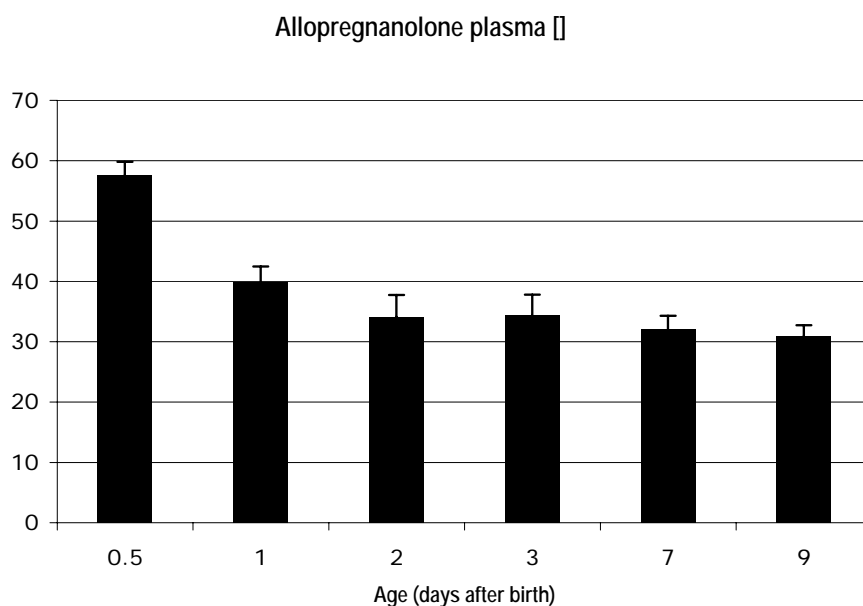


Figure 2: Plasma allopregnanolone concentrations in lambs 0.5 to 9 days after birth.

### 3.5. SUMMARY

The present study measured the concentrations of the neuroinhibitory steroid allopregnanolone in lambs between 12 hrs and 9 days after birth to assess the potential for modulation of conscious perception.

Plasma and brain tissue samples were collected and analysed for allopregnanolone concentrations.

Plasma allopregnanolone concentrations were elevated at 12hrs after birth compared to older lambs. There were no significant changes in allopregnanolone concentrations in any brain region. Thus, it is concluded that if allopregnanolone has the potential to modulate conscious perception in newborn lambs that such modulation would be present at least during the first 12 hours after birth. Further studies will be necessary to elucidate this.

### 3.6. PUBLICATIONS

Manuscript in preparation

### 3.7. REFERENCES

- Barbaccia ML, Roscetti G, Trabucchi M, Ambrosio C & Massotti M. (1992). Cyclic AMP-dependent increase of steroidogenesis in brain cortical minces. *European Journal of Pharmacology* **219**, 485-486.
- Johnson CB, Sylvester SP, Stafford KJ, Mitchinson S, Ward RN & Mellor DJ. (2009). Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen from birth to 6 weeks old. *Veterinary Anaesthesia and Analgesia* **36**, 273-279.
- Mellor DJ & Diesch TJ. (2006). Onset of sentience: The potential for suffering in fetal and newborn farm animals. *Applied Animal Behaviour Science* **100**, 48-57.
- Yawno T, Yan EB, Walker DW & Hirst JJ. (2007). Inhibition of neurosteroid synthesis increases asphyxia-induced brain injury in the late gestation fetal sheep. *Neuroscience* **146**, 1726-1733