

## Reducing mother-young separation distress by inducing ewes into oestrus at the day of weaning

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### Abstract

The aim of this study was to determine whether or not oestrus induction on the day of weaning would reduce the distress experienced by ewes upon separation from lambs. For this, 43 ewes, their eight week-old lambs and six mature rams were used. Prior to weaning, 21 of these ewes were induced to display oestrus on the day of mother-young separation (treated group [T]) while the remaining 22 untreated ewes served as controls (C). T and C ewes were housed together. Blood samples were collected on the day of weaning (prior to mother-young separation and 24 hours later) and then nine days later, for plasma progesterone (P4) and cortisol determination. Lambs were separated from their dams by a wire fence at weaning in full view of each other. Three rams were tethered to posts with 3 m plastic chains in the ewes' pen. T and C groups were simultaneously tested in identical test pens. C ewes showed a greater increase in serum cortisol concentration after separation from lambs ( $47.64 \pm 4.26$  n mol l<sup>-1</sup>) than T ewes ( $28.79 \pm 6.29$  n mol l<sup>-1</sup>). T ewes exhibited fewer vocalisations ewe<sup>-1</sup>h<sup>-1</sup> and fewer vocalisations at 6, 12 and 18 h post separation than C ewes. On the day of weaning, more T ewes were seen to be situated away from lambs and in close proximity to the males, compared to the C group. Thus, more T than C ewes were mounted (14 as opposed to 3) and these received a greater number of mounts ( $1.02 \pm 0.23$  per hour, as opposed to  $0.11 \pm 0.06$ ). It was concluded that by inducing ewes into oestrus at weaning it is possible to reduce the signs of separation distress.

**Keywords:** animal welfare, mother-young separation, proceptivity, sheep, stress, weaning

### Introduction

Weaning of domestic mammals is characterised by the replacement of milk by solid food in association with the breakdown of the mother-young bond. Even though this is a gradual process in nature, which has been reported to occur at six months in Mountain Bighorn Sheep (Geist 1971), four to five months in Merino and Dorset Horn breeds (Arnold *et al* 1979) and at up to 12 months in Soay sheep (Grubb 1974), artificial weaning imposed by the breeder involves mother-young separation and a drastic modification in the feeding habits of the lamb at an early age; generally 50-60 days postpartum in the Pelibuey breeds raised under tropical conditions. This early separation can induce stress in both ewes and lambs (Orgeur *et al* 1998) and is often expressed by an increase in vocalisations and locomotor activity (Alexander 1977; Torres-Hernández & Hohenboken 1979). This stress is also thought to involve neuroendocrine mechanisms with Mormède (1995) suggesting that such social perturbations lead to activation of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system. Oestrous behaviour in sheep is characterised by strong proceptive (ie male-seeking) behaviour (Beach 1976) and physical contact responses

(Beach 1976; Lindsay 1996). Ewes in oestrous will seek out rams and approach and mate with tethered rams (Fletcher & Lindsay 1968; Lindsay & Fletcher 1972; Allison & Davis 1976). Furthermore, ewes in oestrus can compete with other ewes for the attention of the ram (Hulet *et al* 1962; Van der Westhuysen 1971; Tomkins & Bryant 1972).

In this study, we evaluated the effect of inducing ewes into oestrus at the time of mother-young separation. It was hoped this would reduce the distress associated with weaning, by redirecting the ewes' interest toward the rams ie proceptivity, as opposed to the recently separated lamb.

### Materials and methods

This study was carried out at the Instituto Tecnológico de Oaxaca, Mexico, situated 1600 m above sea level with an average annual rainfall of 550 mm and an average annual temperature of 20.4°C.

The subjects were 43 Pelibuey (hair sheep; *Ovis aries*) ewes aged between two and four years, their eight-week old lambs and six intact four-year-old rams. All ewes and lambs were kept intact as a group in an indoor pen until the day of separation. Ewes were fed 600 g of a commercial concentrate per day with 14% protein (Purina, Mexico), and 3 kg

of fresh Taiwan grass per animal, to cover maintenance plus average lactational requirements. Mineral salt and water were offered *ad libitum*, while lambs were given exclusive access to a 16% protein commercial concentrate.

The rams were housed in individual pens approximately 100 m away from the rest of the flock until the day of weaning, and received the same diet as the ewes, with the exception that concentrates were limited to 400 g per day.

Twenty one ewes (T) were induced to exhibit oestrus on the day of mother-young separation by the use of intra-vaginal sponges containing 30 mg flurogestone acetate (Chronogest, Intervet, Mexico DF, Mexico). The sponges were inserted for 12 days and 500-600 iu of PMSG (Folligon, Intervet, Mexico DF, Mexico) was administered (im) at the time of sponge withdrawal. The remaining 22 untreated ewes stayed in the flock and constituted controls (C).

At weaning, lambs were separated from their dams and stationed behind a wire fence at one end of the test pen. At the extreme opposite of the pen, three rams were attached by 3 m plastic chains to aligned posts (Figure 1). T and C groups were tested simultaneously in identical test pens rather than in a common pen to avoid treatment interactions.

No food or water was made available in the pen during the experimental day; a standard procedure adopted during early weaning to reduce the milk production in ewes (Hulet 1980; Ross 1989). All ewes were marked with large numbers to facilitate observation from a distance. The behaviour of individual ewes was recorded using instantaneous sampling (Martin & Bateson 1993) every 60 s in four  $\times$  30 min observation periods: 6, 12, 18 and 24 h post separation. Two people recorded animal activities from an observation walkway, 3 m from the front edge of each pen (Figure 1). Behaviour recorded included proximity ( $\leq$  1 m) of ewes to the fence separating them from the lambs, and the proximity of ewes to the rams (one body length distance from the male). The 1 m and one body length criteria were chosen as they were believed to reflect a strong attraction to either the lamb or the ram in question. The number of mounts received by each ewe and vocalisations emitted were recorded continuously during the four 30 min observation periods.

Ewe blood samples were collected from the jugular vein with vacutainers immediately prior to mother-young separation, 24 hours later and on day nine (all at 0800h). Samples were immediately cooled and held in ice water for a maximum of 40 minutes before plasma separation by centrifugation at 1500 rpm for 15 min.

Serum cortisol concentration was determined in the first two samples to compare the increase in adrenal activity between groups.

Plasma progesterone (P4) concentration was determined for the first sample to ensure that the animals had no functional corpus luteum (CL) in their ovaries at separation (ie the day of oestrus in the treated group). Samples were also taken nine days later because high blood P4 levels would indicate,

retrospectively, that the ewes had been in oestrus during the observation period.

Plasma P4 and serum cortisol concentrations were determined by radioimmunoassay (RIA). Cortisol concentrations were determined in duplicate using commercial coated tube RIA kits (Pantex, Santa Monica, CA) according to the method of Jephcott *et al* (1986). The inter and intra-assay coefficients of variation were 2.02 and 6.2%, respectively. The sensitivity of the assay was 97.1% of the total binding and equal to 0.1 n mol l<sup>-1</sup>. For P4 determination, samples were analysed according to the procedure validated by Rawlings *et al* (1984). The sensitivity of the assay was 0.03 n mol l<sup>-1</sup>. Samples with concentrations of hormones below the assay sensitivity were assigned a concentration equal to the sensitivity of the assay. The samples with mean P4 concentration of 0.42 or 2.36 n mol l<sup>-1</sup>, had intra and inter-assay CVs of 10.3 and 4.8% or 13.7 and 6.0%, respectively. The cut-off value of P4 assays to diagnose the presence of an active corpus luteum was  $\geq$ 1 n mol l<sup>-1</sup>. According to Bartlewski *et al* (1999), mean daily progesterone concentration rises from day 0 to a peak value of 2.92 n mol l<sup>-1</sup> on day 9 post ovulation.

### Statistical analyses

Individual values obtained during the first sample were used as baseline data when analysing changes in cortisol concentration. Treatment differences in cortisol changes between baseline and 24 h later were analysed with a *t*-test (Gill 1978) using Systat (Systat Inc, Aurora, CO) software. Treatment differences in the number of vocalisations and mounts ewe<sup>-1</sup> hour<sup>-1</sup> of observation were analysed using the Wilcoxon Mann-Whitney test (Siegel & Castellan 1988). The Friedman two-way analysis of variance (Siegel & Castellan 1988) containing both treatment and time as factors was used to compare vocalisations ewe<sup>-1</sup> hour<sup>-1</sup> at 6, 12, 18 and 24 h post separation. The chi square test (Siegel & Castellan 1988) was used to compare treatments for the number of ewes mounted and the number of ewes observed to be in close proximity to the rams or lambs on greater than ten separate occasions. This frequency criterion (ten) was established to reflect a strong ram-seeking behaviour or attraction to lambs as opposed to casual, non-directed movement of the ewes.

### Results

Increases in cortisol concentration during the 24 h post weaning were 65% higher in C than in T ewes ( $P < 0.01$ ). ( $47.64 \pm 4.26$  compared to  $28.79 \pm 6.29$  n mol l<sup>-1</sup>). In addition, more T than C ewes (16 versus 3;  $P < 0.05$ ) were observed in close proximity to the rams and more (18 versus 9,  $P < 0.05$ ) were noted away from the lambs.

There were fewer vocalisations in the T group at 6, 12 and 18 hours post separation ( $P < 0.05$ ) and fewer ewes vocalised in the T compared to the C group at six (24% versus 77%), 12 (14% versus 50%), and 18 (5% versus 45%) hours after mother-young separation: (5/21 versus 17/22,  $\chi^2 = 6.54$ , df = 1,  $P = 0.01$ ; 3/21 versus 11/22,  $\chi^2 = 4.57$ , df = 1,  $P = 0.03$  and 1/21 versus 10/22,  $\chi^2 = 7.36$ ,

Figure 1

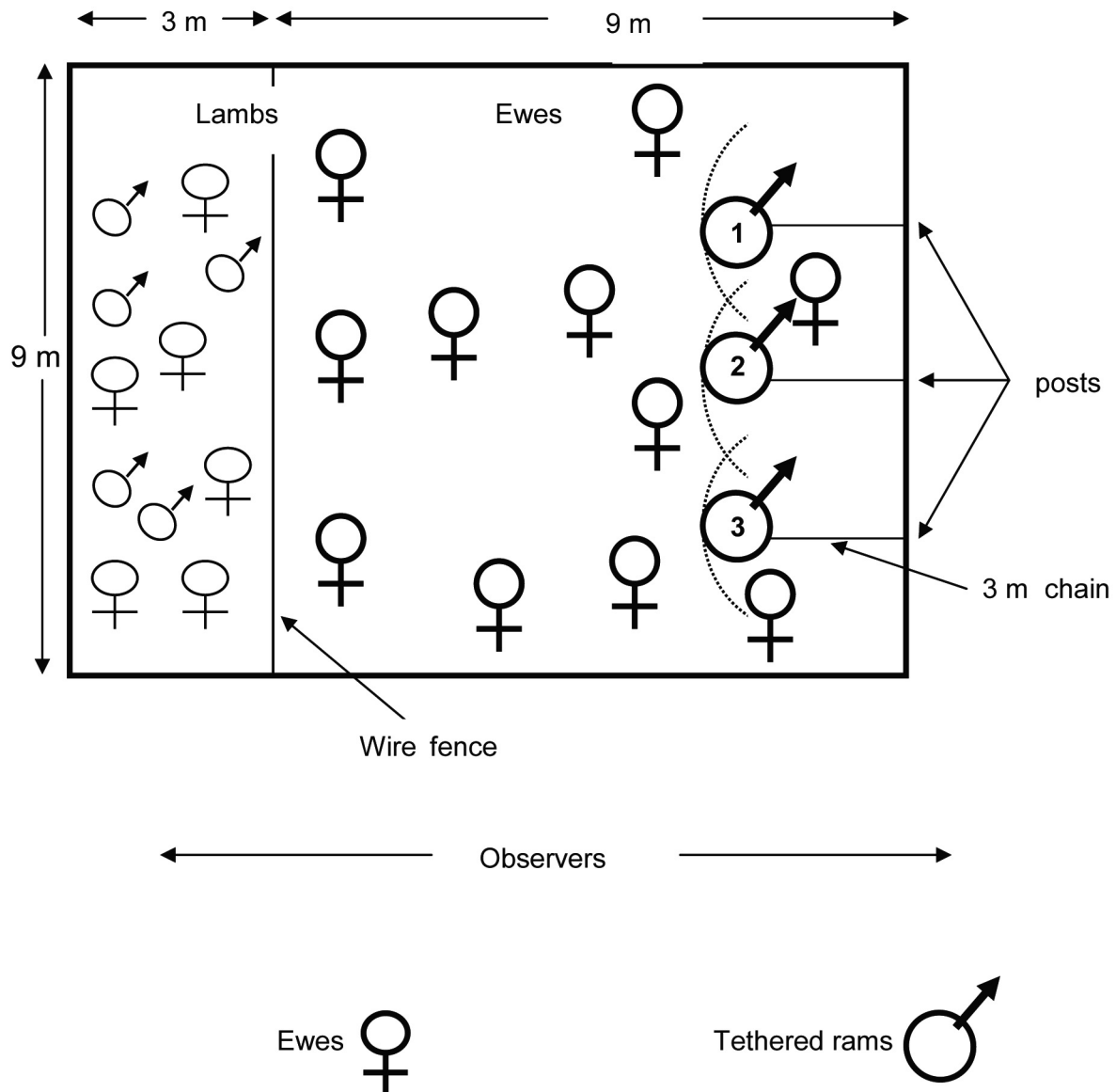


Diagram of the pen used at the day of weaning, establishing the area where the males were tethered and where the lambs were kept separated from their mothers by a wire fence.

df = 1,  $P = 0.01$ , respectively). The same number of animals (7 versus 7) vocalised in both groups 24 hours post weaning (Figure 2).

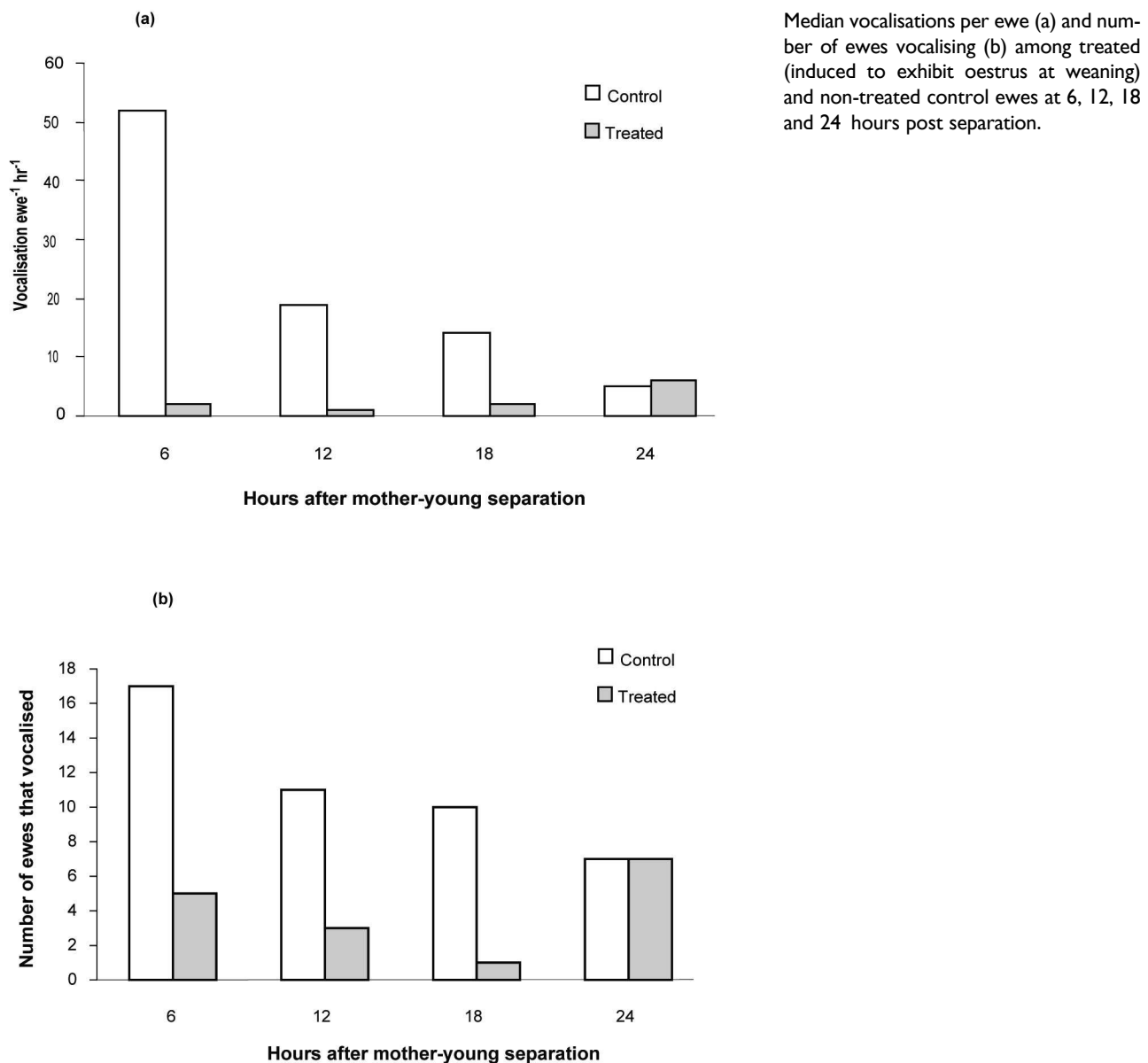
Furthermore, on average, ewes in the T group emitted fewer ( $P < 0.05$ ) vocalisations ewe<sup>-1</sup> hour<sup>-1</sup> of observation ( $12.02 \pm 2.89$ ) than C ewes ( $39.29 \pm 6.63$ ).

More ( $P < 0.05$ ) T than C ewes were mounted (14 versus 3) and a higher ( $P < 0.05$ ) frequency of mounting ( $1.02 \pm 0.23$  versus  $0.11 \pm 0.06$  mounts h<sup>-1</sup> of observation) was observed. These differences were also reflected in the number of ewes seen in close proximity to either lambs or rams, and the

number of T and C ewes mounted during each 6 h observation period (Figure 3).

Immediately following oestrous induction, all T ewes had a P4 concentration below 1 n mol l<sup>-1</sup> and a higher value nine days later. In addition, 67% (14 out of 21) of these ewes exhibited sexual receptivity, most within a 24 h period, starting 12 h after sponge removal. In contrast, 77% (17 out of 22) of the controls had low levels of P4 (< 1 n mol l<sup>-1</sup>) at the day of mother-young separation, and only 13 out of 22 (59%) had P4 concentrations above this level nine days later. Finally, 13% (3 out of 22) of the controls displayed sexual receptivity at the day of mother-young separation.

Figure 2



## Discussion

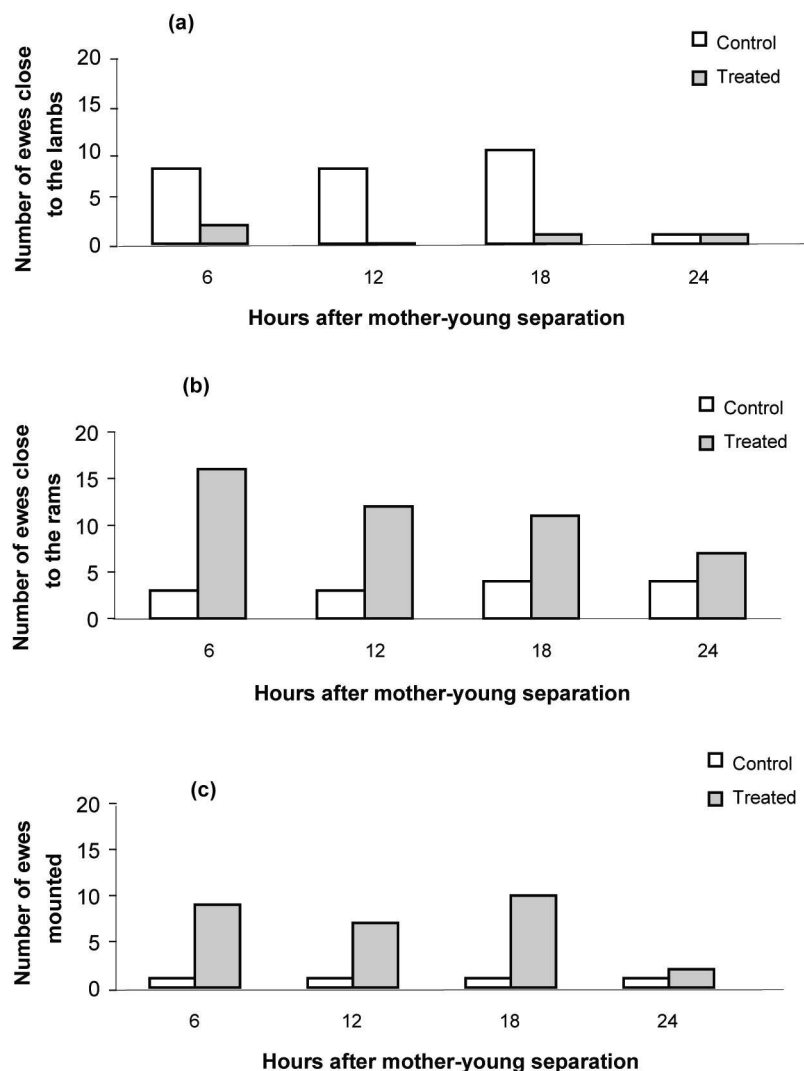
Parrot *et al* (1988) found that pronounced physiological stress increases cortisol levels. Therefore significant differences in cortisol concentration between groups suggest that weaning induces an important stress response in the ewe, even though lambs and ewes were only separated by a wire fence. Orgeur *et al* (1998) compared progressive weaning with daily separation versus sudden weaning, and found that the cortisol concentration of ewes was not affected by separation which ever method was used. In addition, Orihuela *et al* (2004) found similar results after evaluating three weaning practices. This discrepancy can be explained by the fact that the present study had animals subjected to a series of potentially stressful events (ie moving to a new pen, introduction of rams, separation of flock members, food and water deprivation) as well as lamb separation. Nevertheless

all animals (both treatments) were subjected to the same sampling procedure and stressful events. Thus, the differences in the parameters between treatment groups should be due mainly to treatment. The stressful events mentioned above might act synergistically overstating certain values eg cortisol; reflected in higher concentrations than those found in the literature eg 8 to 10  $\text{n mol l}^{-1}$  by Orgeur *et al* (1988).

Ewes and lambs, when separated from each other, typically express their distress by increased bleating and locomotor activity (Alexander 1977; Torres-Hernández & Hohenboken 1979) which is thought to help the animals reunite and cope with this stress-inducing situation. These behavioural changes can persist for the first two days post separation (Orgeur *et al* 1998). In the present experiment, a similar pattern was displayed by the C group in that the number of vocalisations emitted per ewe per hour and the

**Figure 3**

Number of treated and control ewes observed in close proximity to lambs (a) or rams (b), and number of ewes mounted (c) 6, 12, 18 and 24 hours post separation.



number of ewes vocalising, were significantly greater during the first 6 and 12 h post weaning. In contrast, only a slight increase in both variables was observed in the T group during the 24 h observation period immediately after mother-young separation. This may have been induced by the 24 h food and water deprivation associated with the weaning procedure (Hulet 1980; Ross 1989).

Oestrous-induced ewes spent significantly more time than C ewes in close proximity to the rams at weaning, thus decreasing time spent with their lambs. This finding reflects the strong influence of proceptive sexual behaviour in relation to the mother-young bond. The relatively large proportion of time spent by the control ewes in proximity to their lambs is in accordance with other studies, such as Price *et al* (2003) who observed cows spending about 40% of their time within 3 m of the fence separating them from their calves during the first two days following weaning.

The high percentage of ewes that responded to oestrus induction treatment in the present experiment is in keeping with the findings of Avdi and Chemineau (1998) who

reported between 92 and 100% ewes exhibiting oestrous behaviour after artificial oestrous induction. Furthermore, the findings from Godfrey *et al* (1998) reported that the first postpartum oestrus occurred at 39 - 44 days following parturition in St. Croix White hair sheep which could explain the oestrous behaviour observed in some C ewes, as P4 priming is essential for normal patterns of receptive and proceptive behaviours (Fabre-Nys & Martin 1991). In addition, the presence of rams prior to the day of testing might have produced sufficient pheromone effects to cause the stimulation of oestrous behaviours when rams and ewes were brought together in the test pen (Martin *et al* 1986).

Progesterone concentration declines to  $1.10 \text{ n mol l}^{-1}$  by day 14 and reaches a low level of 0.08 on day 16 after ovulation. Consequently P4 concentrations of  $> 1 \text{ n mol l}^{-1}$  were interpreted in the current study to correlate with the presence of an active CL.

The fact that all the animals in the T group had low P4 concentration at the day of weaning and high P4 concentration nine days later, suggests that all animals ovulated, indi-

cating the possibility of high pregnancy rates had ewes been inseminated on the day of weaning. However this hypothesis needs further demonstration. In contrast, 77% of the C group had low P4 concentration on the day of weaning with 59% showing this when tested nine days later; suggesting that a large number of ewes were not cycling at this time.

Studies with hair sheep have observed that 94% of sponge treated ewes exhibit oestrus by 36 h after sponge withdrawal (Godfrey *et al* 1999). Furthermore, Lida *et al* (2004) observed that all ewes showed oestrous behaviour between day 0 and 3 after device removal. These results are in accordance with data from the present study, in which most of the ewes in oestrus displayed sexual receptivity within a 24 h period after sponge removal.

It was concluded that inducing ewes into oestrus at weaning results in a decrease in the occurrence of signs of separation distress.

#### Animal welfare implications

The results of this study support the hypothesis that inducing a shift from maternal to sexual motivation at the day of weaning results in fewer indices of behavioural and physiological distress than in untreated dams. This management technique not only improves animal welfare but may also benefit sheep producers economically by reducing the interval between parturitions in non-seasonal breeding sheep.

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