Effect of transport on pituitary responsiveness to exogenous pulsatile GnRH and oestradiol-induced LH release in intact ewes

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This study examined the effect of transport on GnRH self-priming *in vivo* as well as the consequential effects on the oestradiol-induced LH surge. The follicular phases of ewes (eight per group) were synchronized with progestin sponges, and 50 µg oestradiol benzoate was injected 24 h (time zero) after sponge removal to improve precision in the timing of the LH surge. Beginning 8 h after oestradiol, saline or GnRH (500 ng, i.v.) was given at 2 h intervals with or without 8 h transport beginning 0.5 h before the first GnRH injection (late transport) and effects were compared with those observed during early transport, that is, starting 2.5 h before the first GnRH injection. In all ewes, GnRH alone induced a maximum LH response of 1.9 ± 0.4 ng ml⁻¹ after the first challenge. The response was enhanced ($P < 0.01$) after the second and third GnRH injections (7.4 ± 1.4 ng ml⁻¹ and 7.6 ± 1.7 ng ml⁻¹, respectively). This self-priming effect after the second GnRH was reduced by late transport (7.4 ± 1.4 versus 4.2 ± 0.8 ng ml⁻¹; $P < 0.05$) but not early transport, that is, transport initiated closer to the time of GnRH administration had greater suppressive effects on LH secretion. Throughout transport, spontaneous LH pulse frequency was lower in treated than it was in control ewes (2.38 ± 0.53 versus 4.50 ± 0.53 pulses per 8 h; $P < 0.01$), with marked effects in the first 4 h of transport (1.0 ± 0.19 versus 2.63 ± 0.38 pulses per 4 h; $P < 0.02$). Spontaneous pulse amplitude also tended to decrease during transport (0.13 ± 0.02 versus 0.20 ± 0.03 ng ml⁻¹; $P = 0.07$). When LH turnover was stimulated by exogenous GnRH, the onset of the LH surge was delayed (controls: 20.5 ± 2.0 h versus GnRH alone: 25.3 ± 1.5 h; $P < 0.05$) and the duration was reduced (8.5 ± 0.9 versus 6.5 ± 0.4 h; $P < 0.05$). Transport tended to delay the LH surge in saline-treated ewes (20.5 ± 2.0 versus 22.9 ± 1.9 h; $P = 0.08$), with a further delay imposed by late transport plus GnRH (27.5 ± 1.6 h; $P < 0.05$) but not by early transport plus GnRH (27.8 ± 2.5 versus 26.4 ± 2.4 h; $P > 0.05$), that is, effects mediated by increasing LH turnover were only manifest if transport occurred near the LH surge, when there was insufficient time to replenish stores of releasable LH. In all transported ewes, plasma cortisol increased from 4.5 ± 1.0 ng ml⁻¹ to 29.2 ± 5.5 ng ml⁻¹ ($P < 0.001$) within 15 min of the start of transport and was significantly lower ($P < 0.01$) by 6.5 h. Plasma progesterone also increased from 0.30 ± 0.04 to 0.38 ± 0.04 ng ml⁻¹ ($P < 0.05$). In conclusion, transport affected the oestradiol-induced LH surge by causing a 50% reduction in the self-priming effect of exogenous GnRH, but hypothalamic effects were also revealed by a two-fold decrease in spontaneous LH pulse frequency in saline-treated ewes.

Introduction

Different stressors have deleterious effects on reproduction in laboratory and farm animals (Selye, 1946; Christian, 1960; Moberg, 1987; Dobson and Smith, 1995). These effects are mediated by interference with hormone secretion, expression of oestrus and ovulation (for review, see Phogat *et al.*, 1997a).

The site of action of the stressor effect on gonadotrophin secretion remains unclear. An effect at the pituitary was revealed by suppression of LH release induced by GnRH, for example, during restraint stress in rams (Matteri *et al.*, 1984) or shearing in ewes (Dobson, 1988). An effect at the hypothalamus was also indicated when disturbances of GnRH secretion in ewes were induced by foot shocks (Przekop *et al.*, 1988). While the effects of stressors are probably mediated at multiple sites, the relative importance of each site of action has yet to be established.

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Transport was chosen as a stressor since it is reproducible, of clearly defined but variable duration while the intensity of stimulation remains constant, and associated with well-characterized increases in hypothalamo-pituitary-adrenal activity in sheep (Smith *et al.*, 1997). This stressor reduced the LH response to one injection of GnRH in cows (Dobson, 1987) as well as interfering with the positive feedback effect of oestradiol on surge LH secretion in cows and ewes (Nanda *et al.*, 1990; Smart *et al.*, 1994). After interesting results from experiments *in vitro* (Smart, 1994; Phogat *et al.*, 1997b) in which stress hormones reduced the amount of LH released by multiple exposures to GnRH, we wished to establish that a stressor imposed *in vivo* would interfere with GnRH self-priming during the late follicular phase of the oestrous cycle. Self-priming plays an important role in the control of LH secretion, especially in the late follicular phase before the LH surge (Stelmasiak *et al.*, 1978) and interference with this mechanism may be responsible for stress-induced delays in the LH surge (Dobson and Nanda, 1992).

The effects of transport on LH secretion induced by exogenous GnRH or oestradiol are most marked immediately before the expected time of onset of the LH surge (Dobson and Smith, 1995). All animals in the present study were injected with oestradiol 24 h after synchronization of the follicular phase to reduce the variable timing of the LH surge (Dobson and Nanda, 1992).

Thus, the aim of the present study was to establish the relative importance of stress effects at the hypothalamus or the pituitary in sheep by examining the effect of transport on GnRH self-priming *in vivo*, as well as to elucidate its consequential effects on the oestradiol-induced LH surge.

Materials and Methods

Animals and collection of blood samples

Cross-bred mature intact ewes weighing between 45 and 55 kg were used in a series of experiments during the breeding season. The ewes were loosely penned indoors in pairs, had visual contact with one another and were provided with hay and water *ad libitum*. Before each experiment, the ewes were accustomed to human contact and mock sampling for at least 10 days to minimize the effect of these stressors during the experimental period. Jugular vein catheterization and blood sample processing is described in full by Dobson *et al.* (1999).

Hormone preparations and transport

In each experiment, all ewes were given 50 µg oestradiol benzoate i.m. (Intervet, Cambridge) diluted in 2 ml arachis oil, and GnRH (Fertagyl; Intervet) was given via an indwelling i.v. catheter as a 500 ng injection diluted in 2 ml normal saline. When indicated, groups of 18 ewes were transported untethered (with no physical injuries sustained) in an enclosed truck (4 m × 2 m) along metalled roads for 8 h. During transport, blood samples were collected without the vehicle stopping.

Experimental design

The experimental protocol is summarized (Fig. 1). Oestrous cycles were synchronized with intravaginal progestin sponges (Chronogest; Intervet) inserted for 7 days and, on the day of sponge removal, 250 µg of the synthetic prostaglandin, cloprostenol (Estrumate; Coopers Animal Health Ltd, Sandwich) was given i.m. Twenty-four hours after sponge removal (time 0 h), all ewes were injected with 50 µg oestradiol benzoate. During Expt 1a (control), ewes were divided into saline ($n = 8$) or GnRH ($n = 9$) groups. Beginning 8 h after oestradiol administration, three injections of saline or GnRH were given at intervals of 2 h. Blood samples were collected before sponge removal, before oestradiol injection and, subsequently, starting at 6.5 h, samples were collected at 15 min intervals until 16 h and, thereafter, every 2 h until 40 h after the oestradiol injection. Within this regimen, samples were taken immediately before and 15 min after each GnRH injection.

For Expt 1b (transport), the same regimen was repeated but, in addition, all ewes were transported for 8 h beginning 7.5 h after the oestradiol injection, that is, 0.5 h before the first saline or GnRH challenge. This was termed 'late transport'. After transport, the ewes were returned to the same pens and sampling continued. The same ewes were used for each group to reduce the effect of variation in responses between individuals. Expts 1a and 1b were carried out sequentially because removing animals for transport would disturb control animals that were not transported. This procedure precluded simultaneous experimental grouping of control and transported ewes.

Expt 2 was conducted to examine whether the lower LH response to the second and third GnRH injection in Expt 1 was a reflection of the duration of exposure to transport, hence the period of transport in half the transported ewes was advanced by 2 h but the timing of the GnRH injections relative to the oestradiol injection remained the same. During Expt 2a (control), the protocol of Expt 1a was repeated, except that all ewes ($n = 18$) received three injections of GnRH. The same regimen was carried out during Expt 2b (transport) but for half the ewes ($n = 9$), transport started later, that is, 0.5 h before the first GnRH challenge, whereas for the remaining ewes ($n = 9$), transport started early, that is, 2.5 h before the first GnRH injection. Blood samples were collected before sponge removal, before oestradiol injection and, subsequently, starting at 4.5 h, samples were collected at 15 min intervals until 14 h and, thereafter, every 2 h until 40 h after the oestradiol injection. Within this regimen, samples were taken immediately before and 15 min after each GnRH injection.

Hormone analysis

For each sheep, samples obtained within each experiment were analysed in the same assay. Methods characterized and verified in this laboratory were used to measure LH (Dobson and Ward, 1977), cortisol (Alam *et al.*, 1986) and progesterone (Dobson *et al.*, 1999). For pulsatile LH patterns, samples were analysed using 200 µl plasma and the results were expressed

Fig. 1. Diagram of the experimental protocol: (a) Expt 1a and b; (b) Expt 2a and b. The follicular phases of groups of eight or nine ewes were synchronized with progestin sponges and synthetic prostaglandin (PG). Twenty-four hours later, 50 µg oestradiol benzoate was injected (open arrow) followed 8 h later by three saline or GnRH (500 ng) injections (closed arrows). In Expt 1, late transport (8 h; horizontal bar) began 7.5 h after the oestradiol injection, whereas 9 of 18 ewes began early transport 5.5 h after oestradiol in Expt 2; the remaining nine ewes were transported late. Blood sampling took place every 15 min around the time of transport, reducing to every 2 h thereafter.

as ng equivalents of NIAMDD ovine LH-S21 ml⁻¹ plasma. Interassay and intra-assay coefficients of variation for LH, cortisol and progesterone were both < 9, 9.5 and 7.1%, respectively, with the minimum detectable quantities of 0.3 ng ml⁻¹, 0.4 ng ml⁻¹ and 0.025 ng ml⁻¹, respectively, and precision in the middle range of the standard curve of 0.1, 0.2 and 0.01 ng ml⁻¹, respectively. Measurements of progesterone were unaffected when an amount of cortisol equivalent to 80 ng ml⁻¹ was added to plasma samples containing < 0.5 ng progesterone ml⁻¹, which confirmed the absence of cross-reaction artefacts.

Statistical analysis

Results are expressed as mean ± SEM. The maximum LH response to each bolus of GnRH was calculated by subtracting the basal value (mean of three pre-injection values) from the highest LH value after each GnRH

administration. Data after GnRH injections obtained from control and transport experiments (within the same groups) were compared by ANOVA for repeated measures, followed by comparison of maximum values by Student's paired *t* test, as each sheep served as its own control. However, comparisons among the different groups, within or between the Expts 1 and 2, were made by factorial ANOVA followed by Fisher's test.

The LH pulse data were analysed with the Munro algorithm (Taylor, 1987) as described in detail by Dobson *et al.* (1999). The windows of analysis, which defined the number of samples used to calculate the mean values for periods of comparison, were set at 240 min for the 4 h periods and 480 min for the 8 h periods analysed, as appropriate for each experiment.

Mean LH concentration, pulse amplitude and frequency between control and transported ewes were compared on a within-ewe basis before and during transport using Student's paired *t* test. Values were transformed before statistical

Fig. 2. Effect of 8 h transport on mean ($\pm$ SEM) LH response to three consecutive challenges (500 ng i.v. each at the time indicated by the arrows) given at 2 h intervals when transport (horizontal bar) started (a) late or (b) early in ewes. (a) $\bullet$, no transport ($n = 14$); $\circ$, late transport ($n = 14$). (b) $\bullet$, no transport ($n = 7$); $\circ$, early transport ($n = 7$).

analysis ($\log_{10}$ transformed for hormone concentration and pulse amplitude, and square-root transformed for pulse frequency). For ewes in which the LH surge occurred during the sampling period, LH pulses were considered for statistical analysis only for the period before the onset of the LH surge, and for the same duration in the same ewes in both groups (control and transported).

The occurrence of an LH surge was defined as at least two consecutive samples (at a 2 h interval) containing LH > 5 ng ml $^{-1}$, with the time of first sample > 5 ng ml $^{-1}$ designated as the onset time of the LH surge. The highest value during the LH surge was defined as the surge peak value, and the time between the onset of the surge and the last sample > 5 ng ml $^{-1}$ was used to define the duration of the LH surge.

On 7 of 54 occasions, an early LH surge was provoked during GnRH administration, hence all paired data from these animals were excluded from statistical analysis of GnRH-induced LH secretion. In two ewes during Expt 2b (transport), the LH surge was not detected by the end of sampling, so the time of the last sample was considered as the onset time of the LH surge for the purposes of statistical

analysis. In another ewe, an LH surge was not detected in either Expt 2a (control) or 2b (transport) and, hence, was excluded from the surge analysis.

Results

Data for animals receiving similar treatments did not differ between Expts 1 and 2, and were, therefore, combined for concise description and statistical analysis.

Effect of transport on GnRH self-priming

The first GnRH injection without transport resulted in an increase in LH concentration within 15–30 min, with individual values ranging from 0.5 to 7.1 ng ml $^{-1}$. Maximum values were significantly ($P < 0.01$) enhanced after the second and third GnRH challenge (ranges: 3.9–18.2 ng ml $^{-1}$ and 3.7–23.8 ng ml $^{-1}$, respectively) providing evidence of a self-priming effect (Fig. 2). Values of LH were not altered by injections of saline.

Fig. 3. Direct comparison of the effect of 8 h transport on mean ($\pm$ SEM) LH response to three consecutive GnRH challenges (500 ng i.v. at the times indicated by arrows) given at 2 h intervals when transport (horizontal bar) started early or late in ewes. $\blacktriangle$, early transport ($n = 7$); $\triangle$, late transport ($n = 13$).

Late transport did not affect the peak LH response to the first GnRH injection (1.9 ± 0.4 versus 1.5 ± 0.4 ng ml $^{-1}$; $P < 0.05$; Fig. 2a). However, the peak LH response was significantly decreased by approximately 50% after the second (7.4 ± 1.4 versus 4.4 ± 0.8 ng ml $^{-1}$; $P < 0.01$) and the third challenge (7.6 ± 1.7 versus 4.7 ± 0.6 ng ml $^{-1}$; $P = 0.05$) compared with non-transported controls.

In Expt 2, when transport began early (Fig 2b), there was no effect on the maximum LH response to the first (2.7 ± 0.7 versus 2.0 ± 0.4 ng ml $^{-1}$; $P < 0.05$) or second (4.7 ± 1.3 versus 6.6 ± 0.9 ng ml $^{-1}$; $P < 0.05$) GnRH injection but there was an increased peak LH response to the third GnRH injection (3.7 ± 0.8 versus 7.7 ± 0.9 ng ml $^{-1}$; $P = 0.01$) in the early transported ewes compared with non-transported controls.

Direct comparison of the early and late transport groups treated with GnRH (shown in Fig. 3 for clarity) revealed that the LH response was significantly lower ($P < 0.01$) in the late transported ewes, that is, when GnRH was given soon after the start of transport.

Effect of transport on endogenous pulsatile LH secretion

The patterns of pulsatile LH secretion during saline administration to individual ewes from the control and late transported groups are shown (Fig. 4). Mean LH concentrations and pulse parameters (amplitude and frequency) are also shown (Fig. 5). Mean LH secretion between the two groups did not differ and, although transport only tended to depress mean pulse amplitude ($P = 0.07$), on an individual ewe basis, the pulse amplitude was depressed by transport in five of eight ewes (Fig. 4). Throughout the whole transport period, transport significantly

decreased mean LH pulse frequency (Fig 5), especially in the first 4 h of transport compared with non-transported controls over the same period (1.0 ± 0.19 versus 2.63 ± 0.38 pulses per 4 h; $P < 0.02$) but there were no differences during the second half of transport (1.9 ± 0.1 versus 1.4 ± 0.3 pulses per 4 h).

Effect of transport on the oestradiol-induced LH surge

Within each experiment, the onset of the oestradiol-induced LH surge in individual ewes occurred at variable times ranging from 14 to 38 h after oestradiol administration. The duration and maximum values of the LH surge (4–12 h and 6.2–54.2 ng ml $^{-1}$, respectively) also varied markedly between individuals. However, even without transport, administration of exogenous GnRH delayed the surge onset time by 4.8 h ($P < 0.05$) and reduced the duration by 2 h ($P < 0.05$; Table 1).

The imposition of transport beginning late, but not early, after oestradiol treatment further delayed the onset of the LH surge in GnRH treated ewes, but the delay of 2.4 h caused by transport in saline treated ewes failed to reach statistical significance ($P = 0.08$; Table 1). One sheep in each group was particularly sensitive to stress, with the LH surge being completely blocked by transport whether started late or early after oestradiol treatment. Transport did not affect the duration, or the peak value, of the LH surge ($P > 0.05$) in any of the groups.

The LH surge occurred during GnRH administration on 7 of 54 occasions, including twice in one ewe. The mean basal LH value for all these ewes, before the first GnRH challenge, was 0.2–0.3 ng ml $^{-1}$.

Fig. 4. For legend see facing page.

Effect of transport on plasma cortisol and progesterone profiles

In all transported ewes, plasma cortisol values increased ($P < 0.001$) within 15 min after the start of transport (Fig. 6). Plasma cortisol values decreased from 2.5 h after the start of transport and were significantly lower by 6.5 h into transport ($P < 0.01$). Plasma progesterone concentrations

also increased from 0.30 ± 0.04 ng ml⁻¹ to 0.38 ± 0.04 ng ml⁻¹ during transport, and the area under the curve was significantly higher than it was in non-transported controls ($P < 0.05$; Fig. 6). However, during transport, although the correlation coefficient (r) between cortisol and progesterone in individual ewes ranged from 0.1 to 0.8 (mean $r = 0.27$), the profile of cortisol secretion was not mirrored by that of progesterone.

Fig. 4. Pulsatile LH patterns in control and late transported ewes during administration of saline alone. Individual pulses are depicted by asterisks (*) and the occurrence of an LH surge is shown by arrows.

Discussion

Late transport reduced LH release by interfering with the GnRH self-priming effect. These findings concur with studies *in vitro*, in which 15–30 min pretreatment with adrenocorticotrophic hormone (ACTH) reduced the response to subsequent GnRH challenges (Smart, 1994; Phogat *et al.*, 1997b). *In vivo*, increasing oestradiol concentrations before the occurrence of the LH surge (Gregg and Nett, 1989) and the self-priming action of GnRH (Khalid

et al., 1991) both increase pituitary responsiveness by increasing the number of GnRH receptors. Furthermore, GnRH actions on LH synthesis, storage and transfer from storage to releasable pools before the LH surge are potentiated by oestradiol (Hoff *et al.*, 1977), and there is evidence *in vitro* that ACTH can disrupt oestradiol sensitization of the pituitary (Phogat *et al.*, 1997b). Thus, the adverse effect of transport on LH secretion when gonadotrophin turnover was increased by exogenous GnRH may be mediated through interference with the number of

Table 1. Parameters of the LH surge (mean ± SEM) induced by 50 µg oestradiol (given 24 h after prostaglandin) in ewes with or without 8 h transport beginning late or early after oestradiol administration

	Onset time after oestradiol (h)	Duration (h)	Peak values (ng ml ⁻¹)
Late transport comparison			
Saline alone (n = 8)	20.5 ± 2.0 ^a	8.5 ± 0.9 ^a	32.9 ± 5.2
Saline + Transport (n = 8)	22.9 ± 1.9 ^{ab}	7.2 ± 0.6 ^{xy}	28.5 ± 4.9
GnRH alone (n = 13)*	25.3 ± 1.5 ^b	6.5 ± 0.4 ^y	26.7 ± 4.4
GnRH + Transport (n = 13)*	27.5 ± 1.6 ^c	7.4 ± 0.4 ^{xy}	26.7 ± 3.7
Early transport comparison			
GnRH alone (n = 7) [†]	27.8 ± 2.5 ^{bc}	6.5 ± 0.6 ^y	28.3 ± 4.6
GnRH + Transport (n = 7) [†]	26.4 ± 2.4 ^{bc}	6.4 ± 0.5 ^y	31.8 ± 4.4

*On 5 of 18 occasions (twice in one ewe), an LH surge occurred during the GnRH injection period, and another ewe did not exhibit an LH surge by the end of sampling during Expts 2a (control) and 2b (transport). The paired data from these animals are excluded from this statistical analysis.

[†]Two of nine ewes had an LH surge during the GnRH injection period, hence these paired data are excluded from this statistical analysis.

Within columns, values with different superscripts differ significantly (*P* < 0.05).

Fig. 5. Effect of 8 h transport on pulsatile LH secretion (mean LH, pulse frequency and pulse amplitude) for a period 7.5–16 h during the late follicular phase in ewes after administration of oestradiol 24 h after prostaglandin injection and progesterone withdrawal (a is significantly different from b; *P* < 0.02; Student's paired *t* test). ■, No transport; □, late transport.

GnRH receptors, reduction of a sufficient releasable LH pool, or via post-receptor signal transduction mechanisms involved in LH release (Clayton, 1989).

The later start of transport and, hence, the administration of GnRH closer to the start, caused a greater reduction in the self-priming action of GnRH. This finding indicates that deleterious effects are more pronounced at the time of the initiation of a stressor and that there is a reduced effect later, due possibly to habituation (Rasmussen and Malven, 1983) or to the downregulation of corticotrophin-releasing hormone (CRH) receptors (Canny *et al.*, 1990). The high cortisol values recorded 2.4–4.5 h after the start of transport, compared with lower values 6.5 h later, emphasized the increased hypothalamus–pituitary–adrenal activity in the early stages, substantiated by the immediate secretion of catecholamines, CRH, arginine vasopressin (AVP) and

Fig. 6. Mean (± SEM) plasma cortisol and progesterone concentrations in ewes with or without 8 h transport. ○, Transport (*n* = 17); ●, no transport (*n* = 17).

ACTH (Parrott *et al.*, 1994; Smith *et al.*, 1997). All these stress hormones, individually or in combination, may mediate the effects of transport observed in the present study. There is already evidence *in vitro* for a suppressive effect of CRH and ACTH on LH release (Smart, 1994; Phogat *et al.*, 1997b), along with evidence *in vivo* that the reduction in GnRH-induced LH secretion by ACTH is not mediated by cortisol in sheep (Fuquay and Moberg, 1983; Dobson *et al.*, 1988). It is unclear why early transport appeared to increase the LH response to the third GnRH injection.

An LH surge occurred in four ewes during pulsatile GnRH administration while transport was in progress. Transport without exogenous GnRH in intact ewes cannot influence the onset of the LH surge once the oestradiol 'switch' from negative to positive at the pituitary has occurred (Dobson and Nanda, 1992). Administration of exogenous GnRH can induce an LH surge if this 'switch' has taken place (Clarke, 1995), a notion confirmed by the initiation of the LH surge in three non-transported ewes in the present experiment. However, in ewes that had an LH surge during GnRH administration (with or without transport), there was no indication of an increased pituitary sensitivity to GnRH as basal LH values were not increased immediately before GnRH administration, probably because of increased oestradiol negative feedback at this time.

There is a disparity between the results of the present experiment, in which transport did not reduce the LH response to the first GnRH injection during an exogenous oestradiol re-inforced follicular phase in the breeding season, and an earlier experiment during anoestrus with untreated ewes in which LH secretion after one GnRH injection was significantly reduced by shearing (Dobson, 1988). Apart from the use of a different stressor, which may have caused a different ratio of CRH and AVP release (Canny *et al.*, 1989), the marked steroid-negative feedback in the present experiment may also have masked the effect, thus accounting for the contrasting results.

In the present experiment, an increment in progesterone concentration was observed during the first 2 h of transport. Evidence has been presented to refute any assay artefacts. Progesterone is known to interfere with GnRH secretion from the sheep hypothalamus (Karsch *et al.*, 1987) and there is preliminary evidence to show that increases of 0.9 ng ml⁻¹ in peripheral blood are associated with an instantaneous decrease in oestradiol production by dominant follicles (Noble *et al.*, 1996). It is presumed that progesterone in the present experiment came from the adrenal glands, although, if this is the case, a parallel decrease in cortisol and progesterone through the final hours of transport would have been expected. However, a different secretory pattern from the adrenal gland in response to a stressor may have been responsible for the non-parallel decrease, or this progesterone may not have come from the adrenal gland.

The decrease in LH pulse frequency in transported ewes is in agreement with the LH response to confinement stress in ovariectomized sheep or foot-shock in oestrous sheep (Rasmussen and Malven, 1983; Domanski *et al.*, 1989). In the present study, there was greater suppression of pulse frequency during the first 4 h of transport, which emphasizes the more marked effects at the initiation of a stressor referred

to above. The mean LH concentrations and mean pulse amplitude were unaffected throughout transport, although in terms of results from individual animals, there was an initial reduction in the pulse amplitude in more than half the ewes. The overall effect may have been masked by the marked suppression by the negative feedback of exogenous oestradiol (Caraty *et al.*, 1989). Examination of stress in animals with a reduced steroid milieu may reveal more marked effects.

Transport delayed the onset of the oestradiol-induced LH surge, confirming other reports in cows and sheep (Nanda *et al.*, 1988; Smart *et al.*, 1994). In the present study, the LH surge was delayed in GnRH-treated ewes but the additional effects of transport were only observed when transport started later. It is thought that the negative feedback of oestradiol during the mid-follicular phase in sheep is an obligatory antecedent to an oestradiol-induced, or a naturally occurring, LH surge, allowing a readily releasable pool of LH to accumulate (Blache and Martin, 1995). Exogenous administration of GnRH will deplete LH from this releasable pool; transport may have hindered the replenishment of releasable LH stores. However, the earlier start of transport did not delay the onset of the LH surge, presumably because there was sufficient time to restore the LH releasable pool before the onset of the positive feedback signal to initiate the surge.

The sustained increase of interpulse GnRH concentrations in portal blood during the pre-surge period (as early as 6 h before the onset of the LH surge) may be a vital hypophysiotrophic signal for building up the LH store for surge induction (Evans *et al.*, 1995). Transport during this pre-surge window may perturb the replenishment of the LH store through altered GnRH secretion. Concurrent measurements of GnRH and CRH-AVP during the period of transport-delayed LH surges are required.

In conclusion, during the breeding season, transport affected the oestradiol-induced LH surge by a 50% reduction in the self-priming effect of GnRH, confirming a similar magnitude of effect found *in vitro*. Effects at the hypothalamus were also indicated by a two-fold decrease in spontaneous LH pulse frequency, indicating that hypothalamus-pituitary-adrenal activation has an impact on the hypothalamus-pituitary reproductive axis but effects on pulsatile LH secretion are expressed in different ways. The time of the start of transport relative to the onset of the LH surge was an important factor when considering the consequential effects on the positive feedback effect of oestradiol. Clearly, there are critical windows within the normal feedback process controlling the LH surge, and stressors at these times have deleterious effects depending on the severity of the stressor. If events are so adverse that the oestradiol positive feedback signal is delayed excessively beyond the normal period of GnRH surge release, the LH surge may be completely blocked and ovarian abnormalities will occur that result in subfertility.

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