Effect of transport on pulsatile and surge secretion of LH in ewes in the breeding season

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The aim of this study was to elucidate the mechanism(s) involved in stress-induced subfertility by examining the effect of 4 h transport on surge and pulsatile LH secretion in intact ewes and ovariectomized ewes treated with steroids to induce an artificial follicular phase (model ewes). Transport caused a greater delay in the onset of the LH surge in nine intact ewes than it did in ten ovariectomized ewes (intact: 41.0 ± 0.9 h versus 48.3 ± 0.8 h, $P < 0.02$; ovariectomized model: 40.8 ± 0.6 h versus 42.6 ± 0.5 h, $P < 0.02$). Disruption of the hypothalamus–pituitary endocrine balance in intact ewes may have reduced gonadotrophin stimulation of follicular oestradiol production which had an additional effect on the LH surge mechanism. In the ovariectomized model ewes, this effect was masked by the exogenous supply of oestradiol. However, in these model ewes, there was a greater suppression of maximum LH surge concentrations (intact controls: 29 ± 4 ng ml⁻¹ versus intact transported 22 ± 5 ng ml⁻¹, $P < 0.02$; ovariectomized model controls: 35 ± 7 ng ml⁻¹ versus model transported 15 ± 2 ng ml⁻¹, $P < 0.02$). Subsequent exposure to progesterone for 12 days resulted in the resumption of a normal LH profile in the next follicular phase, indicating that acute stress leads to a temporary endocrine lesion. In four intact ewes transported in the mid-follicular phase, there was a suppression of LH pulse amplitude (0.9 ± 0.3 versus 0.3 ± 0.02 ng ml⁻¹, $P < 0.05$) but a statistically significant effect on pulse frequency was not observed (2.0 ± 0.4 versus 1.7 ± 0.6 pulses per 2 h). In conclusion, activation of the hypothalamus–pituitary–adrenal axis by transport in the follicular phase of intact ewes interrupts surge secretion of LH, possibly by interference with LH pulsatility and, hence, follicular oestradiol production. This disruption of gonadotrophin secretion will have a major impact on fertility.

Introduction

We have established that stress in farm animals reduces fertility (Dobson and Smith, 1995) and the aim of this study was to elucidate further the mechanisms involved. Pulses of GnRH secretion and, hence, LH release are normally controlled by increasing amounts of oestradiol produced by ovarian follicles, eventually leading to the LH surge required for ovulation and luteinization (Goodman, 1994).

The precise timing of a stressor relative to the onset of the LH surge is important. Transport in the 2–4 h period before the onset of the preovulatory LH surge delays the surge, whereas transport 8–10 h before the LH surge has no effect (Dobson and Nanda, 1992; Smart *et al.*, 1994). However, the duration of the spontaneous follicular phase in intact ewes is very variable. Therefore, a model was required in which the stressor could be imposed before a precisely timed LH surge to investigate the effects of transport immediately before this surge. Evans *et al.* (1994) suggested that ovariectomized ewes treated with

progesterone followed by increasing numbers of oestradiol implants provide a repeatable model for the follicular phase in intact ewes. Consequently, the present series of experiments was designed to examine whether transport would cause a delay in the LH surge in ovariectomized, steroid-treated model ewes similar to that observed in intact ewes. Furthermore, as LH is secreted in a pulsatile pattern before the surge, thus forming an important impetus for oestradiol production by the growing follicle (Dobson *et al.*, 1997), the pulsatile nature of GnRH and LH secretion in intact and model ewes during transport was examined.

Materials and Methods

Animals and collection of blood samples

This study was carried out with cross-bred mature ewes weighing between 50 and 75 kg in a series of experiments during the breeding season. In Expt 1, the ewes were housed loosely together in a barn and guided through a handling

race to carry out each procedure. In Expt 2, the ewes were kept indoors in single pens, and had visual contact with one another. In both experiments, concentrate feed was provided twice a day; hay and water were available *ad libitum*.

These experiments began 2 months after the ovaries of half the ewes had been removed under general anaesthesia (a combination of Diazepam ($0.5 \text{ mg kg}^{-1} \text{ i.v.}$; Phoenix Pharmaceuticals, Gloucester) and Ketaset ($5.0 \text{ mg kg}^{-1} \text{ i.v.}$; Fort Dodge Animal Health, Southampton) followed by maintenance on gaseous Halothane (Rhone Merieux, Harlow)) to avoid inflicting pain by transportation on recently ovariectomized animals. A small subcutaneous oestradiol implant ($10 \text{ mm} \times 3.3 \text{ mm}$) was implanted during ovariectomy and remained subcutaneous throughout the study (Ozturk *et al.*, 1998). Artificial oestrous cycles were maintained by administration of luteal concentrations of progesterone using an intra-vaginal device for 10 days (CIDR-G, InterAg, Hamilton; Wheaton *et al.*, 1993). Twenty-two hours after progesterone removal, two large oestradiol implants ($30 \text{ mm} \times 3.3 \text{ mm}$) were inserted subcutaneously, followed 10 h later by a further two 30 mm implants. All the large oestradiol implants were removed 66 h after progesterone removal and a new CIDR-G was inserted to begin the next cycle. Hereafter, ewes treated in this way are referred to as ovariectomized model ewes.

In intact ewes, oestrous cycles were synchronized with a CIDR-G inserted for 10 days and, on the day of progesterone removal, 250 mg of the synthetic prostaglandin, cloprostenol (Estrumate; Coopers Animal Health Ltd, Sandwich), was given intramuscularly. For the cycle immediately before each experiment, two CIDR-Gs were inserted simultaneously for 10 days in intact ewes, because, from previous experience (Kelly *et al.*, 1996), greater synchrony of the LH surge is achieved using two devices rather than one in intact ewes. Only one CIDR-G was used in the ovariectomized model ewes.

Before each experiment, all ewes were accustomed to human contact and mock sampling procedures for at least 10 days to minimize the effect of this stressor during the experimental period. One day before each experiment, indwelling catheters were inserted into the jugular vein under local anaesthesia in all ewes and catheter patency was maintained with heparinized saline (100 iu ml^{-1}). Blood samples (4 ml) were collected in heparinized tubes and immediately centrifuged at 1000 g for 20 min. Plasma was stored at -15°C until analysed.

Transport

In Expt 1, when indicated, groups of 20 ewes were transported untethered in an enclosed truck ($4 \text{ m} \times 2 \text{ m}$) along metalled roads for 4 h. In Expt 2, groups of two or four ewes were restrained in individual metabolism crates ($1.5 \text{ m} \times 0.5 \text{ m}$) to facilitate portal blood sampling. Blood samples were collected during transport without the vehicle stopping. No physical injuries were sustained in either experiment during transport. After transport, the ewes were returned to the original housing and sampling continued. The different parts of each experiment were carried out sequentially because removing animals for transport would

disturb control animals not being transported. In Expt 1, the timing of LH surges in control cycles was monitored before and after the period in which transport occurred to confirm the validity of this procedure. The same ewes were used in each part of each experiment to reduce the effect of variation among individuals. Transport began at the same time of day (noon) in each experiment to avoid any complication of possible diurnal effects.

Experimental design

The experimental protocol is summarized (Fig. 1).

Experiment 1. During Expt 1a (control), ewes were divided into intact ($n = 10$) or ovariectomized model ($n = 10$) groups and monitored during the synchronized, or simulated, follicular phase after progesterone removal. In Expt 1b, the same ewes in the same groups were transported for 4 h from 38 to 42 h after progesterone withdrawal. One oestrous cycle later, in Expt 1c, the protocol for Expt 1a was repeated. In all parts of Expt 1, blood samples were collected at 2 h intervals from 20 to 66 h after progesterone withdrawal.

Experiment 2. The initial aim of Expt 2 was to analyse the effect of transport on GnRH secretion. Consequently, 2 weeks before blood sampling, all ewes had a portal blood collection cannula inserted under ketamine–halothane anaesthesia. Unfortunately, owing to assay difficulties in the laboratory, it was not possible to measure GnRH in the portal samples. However, data were obtained from peripheral LH measurements.

Four intact and three ovariectomized model ewes (chosen from those used in Expt 1 with the closest synchrony of LH surges on repeated occasions) were monitored during control and transported follicular phases (Expt 2a,b), as described in Expt 1, in a cross-over design with more frequent blood sample collection (every 10 min) from 36 to 44 or 48 h after progesterone withdrawal around an early period of transport (38–42 h).

In Expt 2c and d, the same four ovariectomized model ewes were treated as in Expt 2a and b, but the period of transport began 7 h later (that is, from 45 to 49 h after progesterone withdrawal), and blood samples were taken every 10 min from 44 to 60 h.

Hormone analysis

For each sheep, samples obtained within each experiment were analysed in the same assay. Methods characterized and verified in our laboratory were used to measure LH (Dobson and Ward, 1977) and cortisol (Alam *et al.*, 1986). For pulsatile LH patterns, samples were analysed using 200 ml of plasma and the results were expressed as ng equivalents of NIAMDD ovine LH 21 ml^{-1} plasma. Interassay and intra-assay coefficients of variation for LH and cortisol were $<7\%$ and $<7.5\%$, respectively, with minimum detectable quantities of 0.3 ng ml^{-1} and 0.4 ng ml^{-1} , respectively, and precision in the mid-range of the standard curve of 0.1 and 0.2 ng ml^{-1} , respectively.

Experiment 1

Experiment 2

Fig. 1. Summary of experimental protocol after oestrous synchronization with progestin sponges for 10 days (P4). In Expt 1, groups of ten intact or ovariectomized ewes treated with oestradiol implants ($\square$) were transported for 4 h ($\blacksquare$) or acted as controls in the cycle before or after (Expt 1a and c) the transported cycle (Expt 1b). Blood sampling after progesterone withdrawal took place every 2 h as indicated by vertical lines around the time of the expected onset of the LH surge ($\boxtimes$). In Expt 2, groups of three or four ewes, either intact or ovariectomized and treated with oestradiol implants ($\square$), all with a portal sampling apparatus inserted, were transported either early (Expt 2a and b) or late (Expt 2c and d) for 4 h ($\blacksquare$) or acted as controls. Additional portal and peripheral blood samples were taken every 10 min for periods shown ($\boxtimes$). OVX, ovariectomized.

Statistical analysis

Results are expressed as mean $\pm$ SEM. Data obtained from each control experiment and the transport experiment (within the same groups) were compared by ANOVA for

repeated measures, followed by Student's two-tail paired *t* test with significance assigned at $P < 0.05$. The same ewes were used in control and transport experiments within the same groups (and in both Expts 1 and 2 for direct comparison) so the results were compared on a within-ewe

basis, each sheep serving as its own control, to minimize the effects of between-animal variation. However, *post hoc* comparisons between the different groups, within or between Expts 1 and 2, for LH and cortisol were made by an unpaired Student's *t* test.

In Expt 1, the occurrence of the LH surge was defined as at least two consecutive samples (at 2 h intervals) containing > 5 ng LH ml⁻¹, with the time of first sample > 5 ng ml⁻¹ being designated as the onset time of the LH surge. The highest value recorded during the LH surge was defined as the surge maximum, and the time between the first and last sample > 5 ng ml⁻¹ was used to define the duration of the LH surge.

In Expt 2, a presurge baseline was defined as the average of all values in a 5 h period (or as long as available up to 5 h) representative of the presurge secretion for a particular ewe. The onset of the LH surge was defined as the first sample in a series lasting at least 1 h and increasing at least two times above the average of the presurge baseline; this value was always 2 SD above the presurge average.

The LH pulse data from Expts 2a and b were analysed with the Munro algorithm (Taylor, 1987), which is a modified version of the Pulsar algorithm (Merriam and Wachter, 1982). The smoothing windows, which define the number of samples used to calculate the moving averages, were set at 120 min for the 2 h periods and 240 min for the 4 h periods analysed. The nadir and minimum pulse interval windows were each set at 20 min. The rise threshold, the minimum height to which a peak must increase above nadir to be accepted, was set at 1.0 SD. The G parameters (G1–G5) were 3.98, 2.40, 1.68, 1.24 and 0.93; these values give a 5% false positive error rate. The G parameters are the number of SD by which a hormone peak value (composed of one–five samples) must exceed the baseline to be accepted as a pulse. The Baxter parameters, describing the parabolic relationship between the concentration of a hormone in a sample and the SD (assay variation) about that concentration, were 0.013 (b_1 , the y intercept), 0.019 (b_2 , the x coefficient) and 0.003 (b_3 , the x^2 coefficient). The nadir, amplitude and area for each pulse and the mean nadir, mean pulse amplitude, mean pulse area and mean pulse interval for each ewe with or without transport were calculated by the program. Treatment effects,

comparing values within each ewe with or without transport, were identified by Student's paired *t* test. Values were transformed before statistical analysis ($\log_{10}$ transformed for nadir, pulse area, mean concentration and pulse amplitude, and square root transformed for pulse frequency).

Results

LH

Experiment 1. There were no differences in the characteristics of the LH surge between the control experiments carried out before or after the transport experiment (Expt 1a versus 1c; Table 1). In the intact animals, transport for 4 h starting 38 h after progesterone withdrawal resulted in one ewe not having an LH surge observed, and an average 7.3 h delay in surge onset in those ewes that did have a surge (comparing Expt 1a with 1b; Table 1). All the ovariectomized model ewes had an LH surge but the onset was delayed by only approximately 2.0 h. The duration of the LH surge was not affected by transport, but the LH surge maximum was lowered by transport in both intact and ovariectomized ewes, with a greater effect in ovariectomized ewes (average decrease of 7 versus 20 ng ml⁻¹; comparing Expt 1a with 1b; Table 1).

Experiment 2. Insertion of portal collection apparatus in the control groups (intact and ovariectomized model) resulted in later onset of the LH surge after progesterone withdrawal compared with the same animals used in Expt 1 (intact: 41.0 ± 0.9 versus 60.0 ± 6.7 h, $P < 0.01$; ovariectomized model: 40.8 ± 0.6 versus 50.6 ± 1.1 h, $P < 0.05$).

The onset of the LH surge was monitored in the four intact control animals, but sampling stopped before the end of the surge and no further surge data analyses were performed. In two of the three intact animals that were transported in the early period for 4 h starting 38 h after progesterone withdrawal, the onset of the LH surge was not observed before the end of sampling. However, there was no delay in the one animal in which a surge was observed (Table 2).

Table 1. Characteristics (mean $\pm$ SEM) of the LH surge in intact or ovariectomized model ewes with (Expt 1b) or without (Expts 1a,c) transport from 38 to 42 h after progesterone withdrawal

	Time to LH surge onset after progesterone (h)	LH surge duration (h)	Maximum LH (ng ml ⁻¹)	Ewes with LH surge
Intact ewes				
Expt 1a	41.0 ± 0.9^a	8.4 ± 0.9	29 ± 4^c	10/10
Expt 1b	48.3 ± 0.8^b	6.8 ± 1.6	22 ± 5^d	9/10
Expt 1c	40.5 ± 0.9^a	8.3 ± 0.9	32 ± 8^c	9/10
Ovariectomized model ewes				
Expt 1a	40.8 ± 0.6^e	6.4 ± 0.4	35 ± 7^b	10/10
Expt 1b	42.6 ± 0.5^f	5.8 ± 0.7	15 ± 2^h	10/10
Expt 1c	38.4 ± 1.2^e	7.3 ± 0.9	30 ± 6^a	10/10

Within a column for intact or ovariectomized ewes, values with different superscripts differ significantly ($P < 0.02$; Student's paired *t* test).

In one intact ewe in Expt 1b and 1c, an LH surge was not observed by the end of the sampling period, so data from this ewe were excluded from all statistical analysis.

Table 2. Characteristics of the LH surge in intact or ovariectomized model control ewes (Expts 2a and 2c) or those transported from 38 to 42 h (Expt 2b) or 45 to 49 h (Expt 2d) after progesterone withdrawal

	Time to LH surge onset after progesterone (h)	LH surge duration (h)	Maximum LH (ng ml ⁻¹)	Ewes with LH surge
Intact ewes				
Expt 2a (control)	64, 50, 64, 62	Incomplete	Incomplete	4/4
Expt 2b (transport)	50, –, –	Incomplete	Incomplete	1/3
Ovariectomized model ewes				
Expt 2a (control)	50, 50, 52	10, 8, 8	10, 19, 8	3/3
Expt 2b (transport)	50, 52, 48	10, 10, 8	5, 19, 28	3/3
Expt 2c (control)	48, 48, 50, 45	9, 7, 8, 6	40, 28, 75, 13	4/4
Expt 2d (transport)	52, 46, 47, 52	7, 6, 8, 8	13, 4, 75, 5	4/4

Group sizes are small, hence values are shown for individual animals (in the same order each time). Sampling of the intact ewes stopped before the LH surge was complete.

All the ovariectomized model ewes had an LH surge but, because of the small number of animals studied, there was no statistical evidence of a delay in onset when the ewes were transported for 4 h in the early period from 38 h after progesterone withdrawal (Expt 2a,b; Table 2). The other characteristics of the LH surge were also unaltered by transport at this time.

When the ovariectomized model ewes were transported for 4 h in the late period from 45 h after progesterone withdrawal, the timing of the LH surge was delayed by transport in two ewes for 4 and 7 h, respectively, but not in the remaining two ewes. Two of the four animals initiated an LH surge during the period of transport and so it was not possible to assess the effect of transport on pulse characteristics. In three of the four ewes, the LH surge maximum was lower than the surge maximum in non-transported ewes (Fig. 2; Table 2).

Compared with values for the 2 h period before transport, LH pulse amplitude was suppressed in intact ewes by early transport from 38 to 42 h after progesterone withdrawal (0.9 ± 0.3 versus 0.3 ± 0.02 ng ml⁻¹, $P < 0.05$; Table 3, exemplified in Fig. 3), although no effect of transport on pulse amplitude was observed in the ovariectomized model ewes. No effects of transport on pulse frequency were observed in either group of ewes; in intact ewes, there were 2.0 ± 0.4 and 1.7 ± 0.6 pulses per 2 h before and during transport, respectively.

Cortisol

There was a similar marked increase in cortisol secretion during transport in all intact or ovariectomized animals, with no difference in response between the two groups of animal ($P > 0.1$ for area under curve in Fig. 4).

Discussion

Transport for 4 h in the late follicular phase caused a greater delay in the onset of the LH surge in intact ewes than in ovariectomized model ewes (7.3 versus 2.0 h). This finding

Fig. 2. Concentration of LH in the same ovariectomized model ewe with a portal cannula with (a) or without (b) transport (Expt 2c and d) for the time shown (■) in the period after progesterone withdrawal. Pulses are indicated by inverted arrow heads.

indicates that the steroid replacement regimen did not exactly mimic the intact hormonal situation, and that the effects of stress on the hypothalamus–pituitary axis depend on disrupting a very delicate endocrine balance. The steroid milieu provided by the replacement protocol resulted in a more robust stimulus to the hypothalamus–pituitary than that in intact animals (also recognized by Karsch *et al.*, 1980),

Table 3. Mean amplitude ($\pm$ SEM, ng ml⁻¹) and average frequency ($\pm$ SEM, pulses per 2 h) for 2 h before, during and after transport of four intact and three ovariectomized model ewes transported either early (38–42 h after progesterone withdrawal; Expts 2a and 2b) or later (45–49 h after progesterone withdrawal; Expts 2c and 2d)

	Mean amplitude (ng ml ⁻¹)			Mean frequency (pulses per 2 h)		
	Before	During	After	Before	During	After
Intact ewes						
Expt 2a (control)	0.6 $\pm$ 0.2	0.5 $\pm$ 0.3	0.5 $\pm$ 0.1	2.5 $\pm$ 0.6	2.5 $\pm$ 0.2	1.5 $\pm$ 0.3
Expt 2b (transport)	0.9 $\pm$ 0.3	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	2.0 $\pm$ 0.4	1.7 $\pm$ 0.6	2.0 $\pm$ 0.4
Ovariectomized model ewes						
Expt 2a (control)	0.2 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.2	2.0 $\pm$ 0.0	1.5 $\pm$ 0.3	2.7 $\pm$ 0.3
Expt 2b (transport)	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	1.0 $\pm$ 0.6	1.6 $\pm$ 0.6	1.7 $\pm$ 0.3

Transport lasted for 4 h but the above figures have been calculated on a 2 h basis for direct comparison with the 2 h periods before and after transport.

Fig. 3. Concentration of LH in the same intact ewe with a portal cannula with (a) or without (b) transport (Expt 2a and b) for the time shown (■) in the period after progesterone withdrawal.

in spite of peripheral oestradiol concentrations similar to those in intact ewes (Evans *et al.*, 1994). The oestradiol implants deliver a constant supply of steroid, unlike the pulsatile oestradiol pattern that shadows LH pulses in intact ewes (Campbell *et al.*, 1990). If LH pulsatility is disrupted, oestradiol secretion will cease within hours, thus removing the further stimulus for the LH surge (Dobson *et al.*, in press). Thus, stress-induced suppression of LH pulses will contribute to lowered oestradiol concentrations (and hence a marked

Fig. 4. Concentration of cortisol in an intact (a) and ovariectomized (b) ewe with or without transport (□, Expt 1a; ●, Expt 1b; ○, Expt 1c) for the time shown (■) in the period after progesterone withdrawal.

delay in the LH surge) in contrast to the constant supply of oestradiol that will continue from the implants in the model ewes. Consequently, transport disrupted the message for initiation of the LH surge to a greater extent in the intact ewes than it did in the ovariectomized model ewes, although maximum LH surge values were suppressed in both groups. Exposure to progesterone for 12 days after disruption of the LH surge at the end of the follicular phase resulted in a normal LH profile in the subsequent follicular phase.

Therefore, the lesion produced by acute stress in the follicular phase was not permanent.

The presence of the portal blood collection cannula resulted in a delay in the onset of the LH surge in control animals (previously recognized by Moenter *et al.*, 1990, 1991), but there was no firm evidence of a further delay imposed by transport imposed as early as 2 h before the expected onset of the surge. Transport of ovariectomized model ewes nearer the onset of the surge again resulted in lower maximum LH values in animals with this preparation. Although this result could have been due to variable portal lesion sites, this is unlikely as the observation is supported by results from Expt 1, and by similar evidence from Smart *et al.* (1994) that transport reduced the maxima of oestradiol-induced LH surges in postpartum ewes. Kaynard *et al.* (1988) and Evans *et al.* (1995) have established that a precise pattern of GnRH secretion is essential for initiation of a normal LH surge and evidence from LH values in the present study indicates that, if this pattern is interrupted by a stressor, the LH surge will be both delayed and of lower magnitude.

The suppression of LH pulse amplitude in intact ewes transported in the mid-follicular phase concurs with observations in ovariectomized ewes after restraint, insulin-induced hypoglycaemia or endotoxin administration (Rasmussen and Malven, 1983; Clarke *et al.*, 1990; Battaglia *et al.*, 1997). The reduction in amplitude could be due to a direct effect at the pituitary, as there is a reduction in the amount of LH released by GnRH in pituitary tissue exposed to adrenocorticotrophic hormone (ACTH) *in vitro* (Phogat *et al.*, 1997a). However, the reduction in LH pulse amplitude could be indicative of an effect within the hypothalamus resulting in less GnRH being secreted (confirmed by Battaglia *et al.*, 1997), which could also decrease surge peak values, as discussed above, as reducing doses of GnRH depress LH synthesis (Leung *et al.*, 1987; Kaiser *et al.*, 1997). In addition, there is an increase in basal GnRH secretion between pulses, especially 2–4 h before the onset of the LH surge (Evans *et al.*, 1995). It is possible that this basal GnRH is also important for pituitary LH synthesis and that this will be disrupted if GnRH secretion is reduced by stressors.

The present study failed to reveal a statistical effect of transport on LH pulse frequency in the mid-follicular phase, as has been reported by Phogat *et al.* (1999). This result may have been due to the short 4 h period of sample collection before transport (dictated by the portal blood sampling protocol) as well as the discontinuous nature of the pulses that only occurred approximately once per hour.

There was insufficient evidence for a delayed LH surge in the ovariectomized portal model ewes after transport at either 8–12 h or 0–4 h before the expected onset. In fact, in two of the four ewes, the LH surge began during transport, emphasizing the fact that the replacement steroid dose did not replicate precisely the delicate balance that occurs in intact ewes. Oestradiol has direct effects at the pituitary, leading to an LH surge (Kaynard *et al.*, 1988; Clarke *et al.*, 1989). In the ovariectomized model, oestradiol was supplied by the implants and pituitary activation by this, along with pulsatile LH for the previous 24–48 h, may have been sufficient to provoke the initiation of LH surge secretion.

Exactly how LH and GnRH secretion are influenced by

stressors is still not known. Cortisol secretion increased equally in intact and ovariectomized animals in this experiment, and there is evidence for increased corticotrophin-releasing hormone, arginine vasopressin and ACTH secretion during transport (Smith *et al.*, 1997). However, the mechanism(s) by which activation of the hypothalamus–pituitary–adrenal interferes with the reproductive hypothalamus–pituitary axis in sheep is not yet clear (for review, see Phogat *et al.*, 1997b).

In conclusion, transport of intact ewes for 4 h in the hours leading up to the LH surge, reduced pulsatile and surge LH secretion. A reduction in LH pulsatility will lead to poorer follicular development and decreased oestradiol synthesis which, subsequently, will weaken the endocrine messages that initiate the LH surge and, thus, have a major impact on fertility. The effects were not so marked in ovariectomized model ewes to which steroids were constantly supplied by the chosen replacement regimen.

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References

- Alam MGS, Dobson H and Fitzpatrick RJ (1986) Endocrine response to different doses of ACTH in cows *British Veterinary Journal* **142** 239–245
- Battaglia DE, Bowen JM, Krasa HB, Thrun LA, Viguie C and Karsch FJ (1997) Endotoxin inhibits the neuroendocrine axis while stimulating adrenal steroids: a simultaneous view from hypophyseal portal and peripheral blood *Endocrinology* **138** 4273–4281
- Campbell BK, McNeilly AS, Picton HM and Baird DT (1990) The effect of a potent gonadotropin-releasing hormone antagonist on ovarian secretion of oestradiol, inhibin and androstenedione and the concentration of LH and FSH during the follicular phase of the sheep oestrous cycle *Journal of Endocrinology* **126** 377–384
- Clarke IJ, Cummins JT, Jenkin M and Phillips DJ (1989) The oestrogen-induced surge of LH requires a signal pattern of gonadotropin-releasing hormone input to the pituitary gland in the ewe *Journal of Endocrinology* **122** 127–134
- Clarke IJ, Horton RJE and Doughton BW (1990) Investigation of the mechanism by which insulin-induced hypoglycemia decreases luteinizing hormone secretion in ovariectomized ewes *Endocrinology* **127** 1470–1476
- Dobson H (1987) Effect of transport stress on luteinizing hormone released by GnRH *Acta Endocrinologica (Copenhagen)* **115** 63–66
- Dobson H and Nanda AS (1992) Reliability of cyst diagnosis and effect of energy status on LH released by oestradiol or GnRH in cows with ovarian cysts *Theriogenology* **37** 465–472
- Dobson H and Smith RF (1995) Stress and reproduction in farm animals *Journal of Reproduction and Fertility Supplement* **49** 451–461
- Dobson H and Ward WR (1977) Alterations in plasma gonadotrophin patterns caused by sodium pentobarbitone in ewes at oestrus and in anoestrous ewes after infusion of oestradiol *Journal of Endocrinology* **75** 109–118
- Dobson H, Campbell BK and Scaramuzzi RJ (1997) Use of a GnRH antagonist in conjunction with low amplitude, high frequency LH pulses to induce follicular growth without an LH surge and ovulation in ewes *Animal Reproduction Science* **46** 213–222
- Dobson H, Campbell BK, Scaramuzzi RJ and Baird DT Effect of reducing LH pulse frequency and amplitude on ovarian oestradiol production in the ewe *Journal of Reproduction and Fertility Supplement* **54** (in press)
- Evans NP, Dahl GE, Glover BH and Karsch FJ (1994) Central regulation of pulsatile gonadotropin-releasing hormone (GnRH) secretion by estradiol during the period leading up to the preovulatory GnRH surge in the ewe *Endocrinology* **134** 1806–1811
- Evans NP, Dahl GE, Mauger D and Karsch FJ (1995) Estradiol induces both

- qualitative and quantitative changes in the pattern of gonadotropin-releasing hormone secretion during the presurge period in the ewe *Endocrinology* **136** 1603–1609
- Goodman RL** (1994) Neuroendocrine control of the ovine estrous cycle. In *The Physiology of Reproduction* 2nd Edn Vol. 2 pp 659–709 Eds E Knobil and JD Neill. Raven Press, New York
- Kaiser UB, Jakubowiak A, Steinberger A and Chin WW** (1997) Differential effects of gonadotropin-releasing hormone (GnRH) pulse frequency on gonadotropin subunit and GnRH receptor messenger ribonucleic acid levels *in vitro*. *Endocrinology* **138** 1224–1231
- Karsch FJ, Legan SJ, Ryan KD and Foster DL** (1980) Importance of estradiol and progesterone in regulating LH secretion and estrous behaviour during the sheep estrous cycle *Biology of Reproduction* **23** 404–413
- Kaynard AH, Malpoux B, Robinson JE, Wayne NL and Karsch FJ** (1988) Importance of pituitary and neural actions of estradiol in induction of the luteinizing hormone surge in the ewe *Neuroendocrinology* **48** 296–303
- Kelly VM, Phogat JB, Smith RF, Deacon SE and Dobson H** (1996) Pre-treatment progesterone dose on the timing of the LH surge in ewes *Acta Neurobiologiae Experimentalis* **56** 845
- Leung K, Kaynard AH, Negrini BP, Kim KE, Maurer RA and Landefeld TD** (1987) Differential regulation of gonadotropin subunit messenger ribonucleic-acids by gonadotropin-releasing hormone pulse frequency in ewes *Molecular Endocrinology* **1** 724–728
- Merriam GR and Wachter KW** (1982) Algorithms for the study of episodic hormone secretion *American Journal of Physiology* **243** E310–E318
- Moenter SM, Caraty A and Karsch FJ** (1990) The estradiol-induced surge of gonadotropin-releasing hormone in the ewe *Endocrinology* **127** 1375–1384
- Moenter SM, Caraty A, Locatelli A and Karsch FJ** (1991) Pattern of gonadotropin-releasing hormone (GnRH) secretion leading up to ovulation in the ewe – existence of a preovulatory GnRH surge *Endocrinology* **129** 1175–1182
- Ozturk M, Smith RF and Dobson H** (1998) Effect of prolonged exposure to oestradiol on subsequent LH secretion in ewes *Journal of Reproduction and Fertility* **114** 1–9
- Phogat JB, Smith RF and Dobson H** (1997a) Effect of adrenocorticotrophic hormone on gonadotrophin releasing hormone-induced luteinizing hormone secretion *in vitro*. *Animal Reproduction Science* **48** 53–65
- Phogat PB, Smith RF and Dobson H** (1997b) The influence of stress on neuroendocrine control of the hypothalamic–pituitary–ovarian axis *Veterinary Bulletin* **67** 553–567
- Phogat PB, Smith RF and Dobson H** (1999) Effect of transport on pituitary responsiveness to exogenous pulsatile GnRH and oestradiol induced LH release in intact ewes *Journal of Reproduction and Fertility* **116** 9–18
- Rasmussen DD and Malven PV** (1983) Effects of confinement stress on episodic secretion of LH in ovariectomised sheep *Neuroendocrinology* **36** 392–396
- Smart D, Forhead AJ, Smith RF and Dobson H** (1994) Transport stress delays the oestradiol-induced LH surge by a non-opioidergic mechanism in the early postpartum ewe *Journal of Endocrinology* **142** 447–451
- Smith RF, Gore SW, Phogat JB and Dobson H** (1997) The psychological stress of transport stimulates both CRF and AVP secretion into hypophyseal portal blood *Journal of Endocrinology Supplement* **152** 172
- Taylor PL** (1987) *Munro Hormone Pulse-profile Analysis* Elsevier, Amsterdam
- Wheaton JE, Carlson KM, Windels HF and Johnston LJ** (1993) CIDR – a new progesterone-releasing intravaginal device for induction of estrus and cycle control in sheep and goats *Animal Reproduction Science* **33** 127–141