Ovulation rate and the concentrations of gonadotrophins and metabolic hormones in ewes infused with glucose during the late luteal phase of the oestrous cycle

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Abstract

The positive relationship between nutrition and ovulation rate was investigated in sheep infused intravenously with glucose. Ovulation rate increased (2·0 ± 0·0 vs 2·4 ± 0·3) when ewes were given an infusion of glucose (60–65 mM/h) for five days in the late luteal phase of the oestrous cycle. The effect of glucose was obtained without any significant change in LH secretion. The concentration of FSH in glucose-infused ewes was lower during the infusion (luteal phase) but higher during the early follicular phase. These data suggest that the change in ovulation rate occurred without increased gonadotrophin support to the follicle during the late luteal phase, which is the period of the sheep oestrous cycle during which improved nutrition increases ovulation rate. There were no changes in GH or prolactin, but changes in circulating glucose and insulin levels were detected. We conclude that insulin, because of its role in cell growth and metabolism, is involved in mediating ovulation responses to nutritional stimuli, either directly or more likely by the stimulation of insulin-mediated glucose uptake.

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Introduction

Nutritional effects on female gonadal function are well known (Lindsay *et al.* 1991). Nutritional restriction is associated with anovulation whereas high nutrient availability is associated with increased prolificacy.

As early as 1934, Clark reported a positive correlation between the consumption of a high grain diet and ovulation rate in ewes (Clark 1934). It was later suggested that the high energy content of the grain was the stimulus for the increase in ovulation response (Howland *et al.* 1966). In gilts there is a positive relationship between dietary energy level and ovulation rate (Anderson & Melampy 1972, Cole 1982). The infusion of two types of protein or glucose into the abomasum of ewes, increased the percentage of ewes with multiple ovulations (Cruickshank *et al.* 1988) and the peripheral infusion of energy-yielding substrates such as glucose and acetate also increased ovulation rate (Teleni *et al.* 1989*a*,*b*).

On the other hand hypoglycaemia is associated with reduced fertility in cattle (Oxenreider & Wagner 1971, Patil & Despande 1979, Rutter & Manns 1987) and nutritional anoestrus can be induced by restricting energy intake (Imakawa *et al.* 1986, Johnson *et al.* 1987). It has been proposed that under conditions of energy deficit, hypothalamic control of pituitary gonadotrophin secretion may fail because of an inadequate glucose supply (Lynn *et al.* 1965, McClure 1968, Sen *et al.* 1979, Rutter *et al.* 1983, Downing & Scaramuzzi 1993). Nutritional anoestrus in cattle has also been attributed to decreased gonadotrophin secretion (Humphrey *et al.* 1983, Imakawa *et al.* 1986) and, in particular, decreased luteinizing hormone (LH) pulse frequency (Richards *et al.* 1989).

Plane of nutrition may act remotely at the hypothalamus to affect pituitary function or directly at the ovary to affect ovarian function or it could act at both levels. Indeed it is possible that nutrition acts on the hypothalamo-pituitary axis to affect fertility (anovulation) and directly on the ovary to affect prolificacy (ovulation rate). The aim of the present study was to investigate the effect of glucose infusion into the peripheral circulation of ewes during the late luteal phase on ovulation rate and the circulating levels of gonadotrophins and metabolic hormones. The late luteal phase was selected because nutritional effects on ovulation rate can be reliably reproduced at this time (Downing 1994, Downing *et al.* 1995)

Materials and Methods

Animals and diet

Twenty three Border Leicester × Merino ewes, 5 to 6 years of age, were used. During the course of the

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experiment the ewes were housed in single pens in an open-sided shed and had free access to fresh water at all times.

For 7 days the ewes were fed, *ad libitum*, a diet consisting of 4 parts by weight ground oaten straw supplemented with minerals (Downing 1994, Downing *et al.* 1995) plus 1 part by weight chopped lucerne. For the remainder of the experiment all ewes were fed the straw alone. The day on which straw feeding commenced was designated as day 0 of the experiment. The oaten straw diet contained 94·4% dry matter and 1·9% crude protein. The dry matter digestibility and the protein digestibility were estimated in a group of 6 ewes (Downing 1994) at 41·1 ± 1·5% and 44·4 ± 2·4% (mean ± S.E.M.) respectively.

Daily feed intake was measured over 24 days and the ewes were weighed before their daily feeding on days −4, 5, 9, 12, 19, 31 and 36 relative to the day on which the feeding of the straw diet was commenced.

Experimental design

Oestrus was synchronized with two injections of 125 μg Cloprostenol, an analogue of prostaglandin $F_{2\alpha}$ (PG; Estrumate; ICI Pty Ltd, Sydney, NSW, Australia), the first on day 4 and the second on day 15. The experimental sampling began on day 25, that is day 8 of the oestrous cycle that followed the second injection of PG.

On day 24 (day 7 of the oestrous cycle, that is 9 days after the second PG injection) all ewes were fitted, under local xylocaine anaesthesia, with indwelling venous catheters in each jugular vein. One catheter was used for infusion and the other for blood sampling. When not in use the catheters were filled with a solution of sterile heparinized saline (50 iu/ml). The ewes were given daily prophylactic antibiotic treatment (4 ml intra-muscular; Depomycin, Intervet Aust. Pty Ltd, Lane Cove, NSW, Australia).

On the day following cannulation (day 25), starting at 1700 h, all ewes were infused with physiological saline through one of the jugular venous catheters. On the next day, 12 ewes were each infused with 60–65 mM glucose/h for 5 days (from 1000 h on day 26 through to 1000 h on day 31). The remaining 11 ewes continued on saline infusion.

At the end of the infusion period (day 31) all ewes were given a third prostaglandin injection and nine days later (day 40) the ovulation number was determined by endoscopy carried out under xylocaine-induced local anaesthesia.

Blood samples (10 ml) were taken by venepuncture, 2 to 3 times per week from day 0, for the estimation of plasma progesterone concentrations. On the first day of the infusion period, when all ewes were infused with saline, samples of blood (3 ml) were taken every 4 h until 52 h after the end of the infusion. This long period of 4-hourly sampling was interrupted on the third day of the glucose infusion by a 24-h period of more intensive sampling (3 ml every 20 min starting at 1000 h). Apart from the samples collected by venepuncture prior to cannulation, all samples were collected from the indwelling jugular venous catheter. Heparin (100 iu/tube) was used as an anticoagulant. The blood was centrifuged and the plasma removed and stored at −20 °C.

Assays

All assays were based on previously validated assays and complete details of the antisera, the assays and their re-validation are given elsewhere (Downing 1994, Downing *et al.* 1995).

Progesterone concentrations were determined (Downing 1994, Downing *et al.* 1995) in the samples collected 3 times a week. The intra-assay coefficient of variation was 8·5%, the interassay coefficient of variation was 11·9% and the sensitivity of the assay was 0·16 μM.

All of the plasma samples collected over the 24-h period of intensive sampling were assayed for LH (Scaramuzzi *et al.* 1970, Downing *et al.* 1995). The intra-assay coefficient of variation was 9·4%, the interassay coefficient of variation was 11·9% and the sensitivity of the assay was 0·2 μg/l.

Samples collected at 4-hourly intervals were also assayed for follicle-stimulating hormone (FSH) (Downing 1994, Downing *et al.* 1995), growth hormone (GH) (Downing 1994, Downing *et al.* 1995) and prolactin (Downing 1994, Downing *et al.* 1995). The intra-assay coefficients of variation were 5·8%, 9·2% and 7·8%, the interassay coefficients of variation were 10·9%, 9·9% and 9·1% and the sensitivities of the assays were 0·07 μg/l, 0·45 μg/l and 1·6 μg/l for FSH, GH and prolactin respectively.

Plasma insulin concentrations (Downing 1994, Downing *et al.* 1995) were determined in hourly samples collected between 0 and 4 h and between 18 and 22 h after feeding on the third day of glucose infusion. The intra-assay coefficient of variation was 5·7%, the interassay coefficient of variation was 5·2% and the sensitivity of the assay was 0·09 μg/l.

Glucose concentrations were determined (Werner *et al.* 1970) in samples taken at −24, 4, 24, 48, 72, 96, 144 and 168 h relative to the start of the glucose infusion.

Statistical analyses

Differences in ovulation rate were compared by a Chi-squared test of independence utilizing categories of 1, 2 or 3 ovulations. Differences in mean live weights were compared by an unpaired *t*-test. The values for the parameters of pulsatile LH release during the intensive bleed on day 3 of the glucose infusion were estimated using the 'Munro' pulse analysis program (Zaristow Software, Haddington, E Lothian, UK). Comparisons of the pulse parameter values were made by unpaired *t*-tests.

TABLE 1. The body weight (kg; mean ± S.E.M.) of ewes fed an oaten straw diet and infused with either saline (control) or glucose for 5 days in the late luteal phase of the oestrous cycle

	Control	Glucose
Time relative to start of straw feeding (days)		
−4	62·4 ± 1·7	63·7 ± 2·2
5	60·9 ± 1·5	61·5 ± 2·2
9	61·1 ± 1·5	62·1 ± 2·2
12	61·4 ± 1·5	61·9 ± 2·2
19	59·1 ± 1·5	59·9 ± 2·2
31	54·8 ± 1·5	55·3 ± 2·2
36	54·9 ± 1·5	55·4 ± 2·0

The effects of time and of treatment and their interaction were analysed by a repeated measures analysis of variance (CLR, ANOVA; Clear Lake Research Incorporated, Houston, TX, USA) for feed intake and the plasma concentrations of glucose, insulin, FSH, GH and prolactin. The effect of treatment at given times was tested using a pooled error term.

Results

The plasma progesterone profiles of two ewes in the saline-infused control group indicated that they had early luteal regression, one starting on day 2 and one on day 4 of the glucose infusion period. The data from these two ewes were not included in the analyses, leaving group sizes of 9 for saline-infused control ewes and 12 for the glucose-infused ewes.

Ovulation rate

The mean ovulation rate of glucose-infused ewes was 2·4 ± 0·3 and that of the saline-infused ewes was 2·0 ± 0·0 (P=0·053).

Live weight

There were no significant differences between the two groups at any time although there was a significant decline (P<0·05) in both groups over the course of the study (Table 1).

Feed intake

The mean daily intakes of straw over the 24-day study period are shown in Fig. 1. Both groups of ewes ate similar amounts of straw during the 12 days before glucose infusion. During the 5 days of the infusion the glucose-infused ewes consumed significantly less straw (485 ± 27 vs 741 ± 12 g/day, mean ± S.E.M.; P<0·001). By the third day after the end of the infusion, the straw intake of the glucose-infused ewes had returned to levels seen in the saline-infused ewes.

Glucose

The plasma concentrations of glucose at various times relative to the start of infusion are shown in Fig. 2. The concentration in the saline-infused ewes was controlled within very precise limits as can be seen from the small standard errors. The day-to-day variation was also low with the mean for the control group varying by only

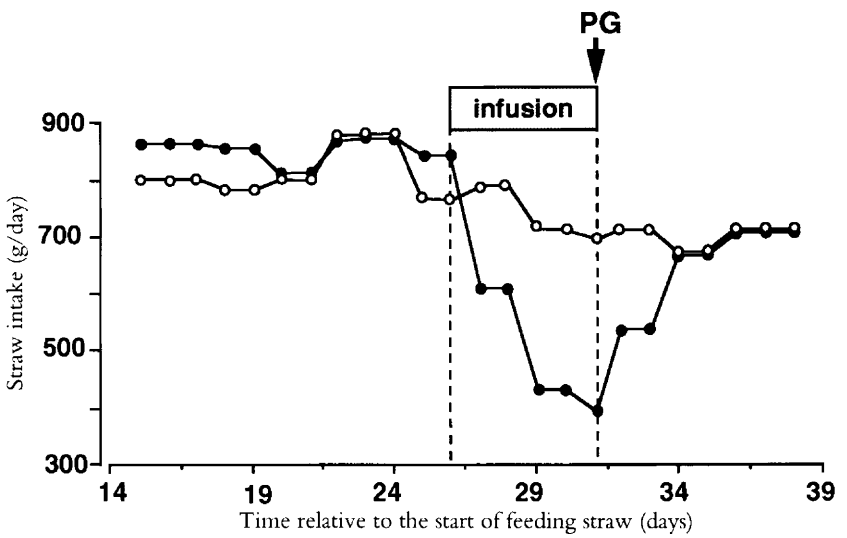

FIGURE 1. The mean daily feed intake in ewes fed an oaten straw diet and infused with either saline (○) or glucose (60–65 mM/h; ●) for 5 days in the late luteal phase of the oestrous cycle.

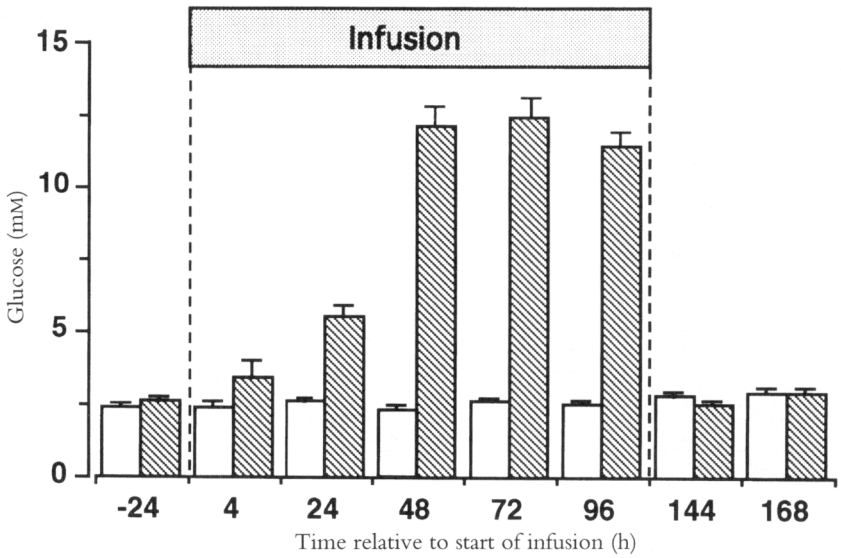

FIGURE 2. The concentrations of glucose in plasma from ewes fed a diet of oaten straw and infused with either saline (open bars) or glucose (60–65 mM/h; shaded bars) for 5 days during the late luteal phase of the oestrous cycle.

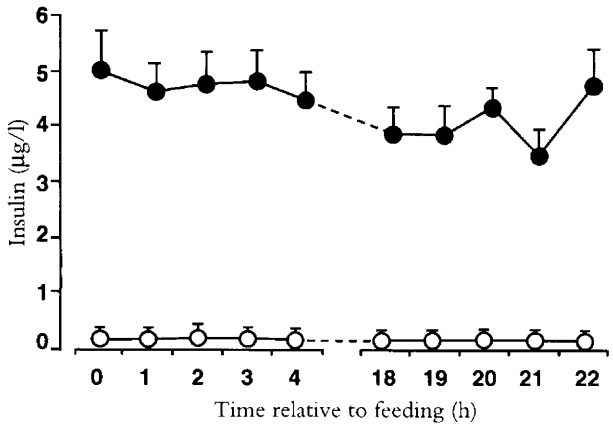

FIGURE 3. The mean (± S.E.M.) plasma insulin concentrations, relative to the time of feeding on the third day of glucose infusion, in ewes fed a basal diet of oaten straw and infused with either saline (○) or glucose (60–65 mM/h; ●) for 5 days in the late luteal phase of the oestrous cycle. The two sampling periods were at 47 to 51 and 65 to 69 h after the start of the glucose infusion.

0·55 mM over 7 days. Within 24 h from the start of the glucose infusion the mean (± S.E.M.) plasma concentration of glucose had increased to 5·7 ± 0·4 mM, with peak values of greater than 11 mM over the last three days of the infusion period.

Insulin

Mean insulin concentrations were significantly ($P<0·001$) greater in glucose-infused ewes compared with saline-infused control ewes (Fig. 3).

TABLE 2. The mean (± S.E.M.) values for the LH pulse analysis in ewes fed a diet of oaten straw supplemented with minerals and infused with either saline (control) or glucose for 5 days in the late luteal phase of the oestrous cycle. Blood was sampled at 20-min intervals on the third day of glucose infusion

	Control	Glucose
Variable		
Pulses/24 h	3·2 ± 0·4	3·7 ± 0·3
Interval (min)	437 ± 64	436 ± 44
Amplitude (μg/l)	1·6 ± 0·3	1·6 ± 0·4
Nadir (μg/l)	0·34 ± 0·04	0·23 ± 0·02*
Mean level (μg/l)	0·54 ± 0·06	0·38 ± 0·07

*$P<0·05$ compared with control values.

Luteinizing hormone

The details of the LH pulse analysis are presented in Table 2. Only the nadir of LH was significantly affected by glucose infusion ($P<0·05$).

Follicle-stimulating hormone

The FSH levels in the saline-infused ewes remained relatively constant until the time of the PG injection (Fig. 4). The difference in the mean levels over the pre-treatment period and for the first 60 h of the infusion were not significant between groups. At about 64 h after the start of the infusion the mean FSH concentrations in the glucose-infused ewes declined and were consistently lower over the period 64 to 120 h after the start of the glucose infusion. The FSH concentrations from 84 to 104 h and at 120 h after the start of the infusion were significantly lower in the glucose-infused group of ewes ($P<0·05$).

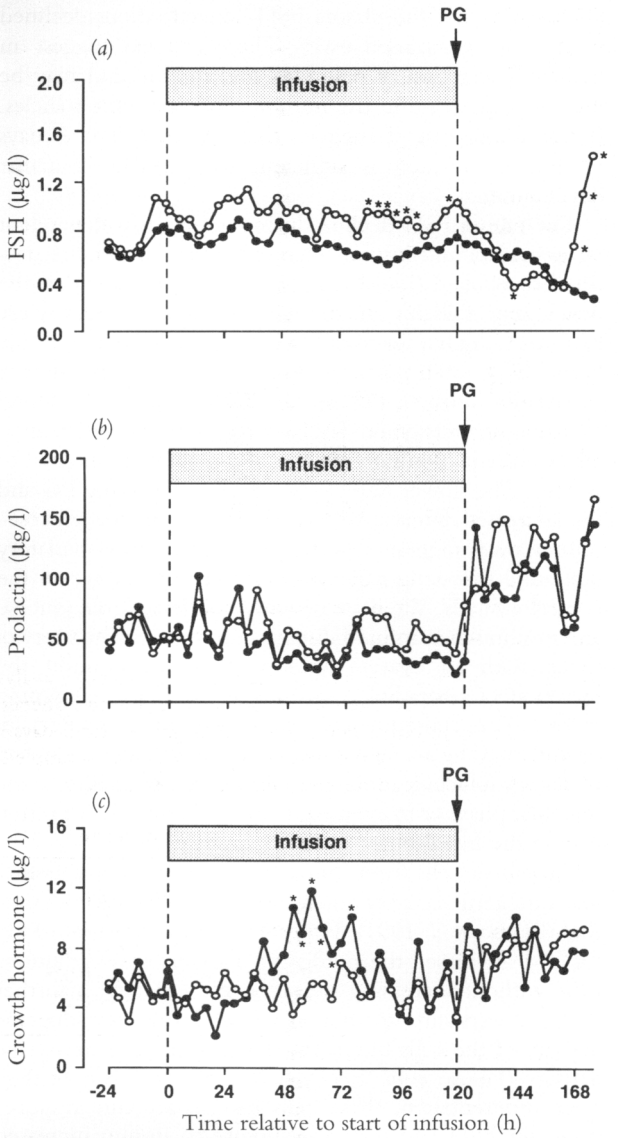

FIGURE 4. Concentrations of plasma (*a*) FSH, (*b*) prolactin and (*c*) GH in ewes fed an oaten straw diet and infused with saline (○) or glucose (60–65 mM/h; ●) for 5 days in the late luteal phase of the oestrous cycle. The time of PG injection is indicated. For clarity, the standard errors have not been added. Values marked with * are different at $P<0{\cdot}05$.

Following the PG injection at the end of the infusion period, FSH levels in the saline-infused ewes began to decline and reached their lowest point around 24 h post-PG injection ($P<0{\cdot}05$). The FSH levels in the glucose-infused ewes also fell after the PG injection reaching their lowest point at the end of the experiment (52 h). Over the period 20 to 28 h post-PG injection, the FSH concentrations tended to be higher in the glucose-infused ewes (significantly higher at 24 h). In the saline-infused ewes there was a rapid increase in plasma FSH at around 40 h after the PG injection. This was because 5 of the 9 ewes in this group had an FSH surge. None of the glucose-infused ewes showed any evidence of an FSH surge during the 56 h post-PG.

Prolactin

There were no significant differences in the mean plasma prolactin concentrations between control and glucose-infused ewes (Fig. 4). There was a significant effect of time but no significant time × treatment interaction. Following PG injection, there was an approximately 3-fold increase in prolactin concentrations for both groups.

Growth hormone

The GH concentrations were higher in the glucose-infused ewes over the period 48 to 72 h from the start of the infusion but this difference was only significant over the period 52 to 68 h after the start of the infusion (Fig. 4). There was no significant treatment effect on the mean plasma concentrations of GH. There were significant time effects and also a significant time × treatment interaction. An increase in growth hormone concentrations in both groups was observed following the PG injection given at the end of the infusion period. The mean plasma growth hormone levels for the last 24 h of the infusion were $5{\cdot}4 \pm 1{\cdot}1$ μg/l for the ewes infused with glucose and $5{\cdot}7 \pm 0{\cdot}7$ μg/l for the ewes infused with saline. For the first 24 h after PG injection the mean levels increased to $9{\cdot}2 \pm 1{\cdot}0$ μg/l and $8{\cdot}8 \pm 0{\cdot}8$ μg/l for the glucose- and saline-infused ewes respectively.

Discussion

Infusion of glucose for 5 days during the late luteal phase of the oestrous cycle was associated with an increase in ovulation rate. These data agree with other reports in which the infusion of glucose or acetate for 9 days prior to ovulation increased ovulation rate in ewes (Teleni *et al.* 1989*a*,*b*). Abdalla *et al.* (1986) reported that infusion of glucose increased the glucose entry rate in ewes. Sixty three percent of the variation in ovulation rate of ewes given various nutritional treatments was associated with variation in glucose entry rate (Rowe 1986). An increase in glucose uptake by the ovary may be the link between nutrition and ovulation rate, and the increased availability of energy may be a critical component of nutritionally induced increases in ovulation rate.

The significant decline in live weight seen in both groups over the experimental period is a result of the low levels of digestible energy and protein in the basal diet. The daily intake of straw also declined in the saline-infused ewes probably due to a slow rate of digestion, as a consequence of the low level of available nitrogen

in the diet. Glucose infusion resulted in an additional decline in the intake of straw (Rutter & Manns 1986). The maintenance of high glucose levels in this group could be responsible as brain areas regulating hunger and satiety in monogastric animals are sensitive to high circulating concentrations of glucose (Ritter 1984, Schwartz *et al.* 1992) but the evidence for such an effect in ruminants is equivocal (Baile & Della-Ferra 1981).

There was no effect of glucose on the overall concentration of GH, although concentrations were significantly elevated for a short period during the infusion. These changes might have been related to the decreased straw intake in the glucose-infused ewes. While the amount of total energy available is probably greater, the decrease in protein digested could be responsible for the changes in GH. The concentration of GH in the plasma is negatively correlated with the amount of protein digested in the alimentary tract of sheep (Bassett *et al.* 1971). A 12-h infusion of GH into ewes with an autotransplanted ovary has been shown to stimulate oestradiol secretion (Downing *et al.* 1993*a*, Downing 1994), but the effect, if any, this short period of elevated GH might have on the reproductive system is difficult to assess in this experiment.

The infusion of glucose had no significant effect on the LH pulse frequency or on other LH pulse characteristics except for the nadir which was depressed. The infusion of glucose into rams also had no effect on LH pulse frequency (Boukhliq *et al.* 1991). Low levels of energy intake suppress LH release in cattle (Humphrey *et al.* 1983, Imakawa *et al.* 1986) and gilts (Armstrong & Britt 1987, Flowers *et al.* 1989) and the induction of hypoglycaemia, with insulin, decreases the frequency of pulsatile LH release in ewes (Downing & Scaramuzzi 1993). These reports suggest that when glucose supply is inadequate, the hypothalamic control of pituitary gonadotrophin secretion may fail. However, as these data show, it does not necessarily follow that an increase in the glucose supply will have the opposite effect. When energy intake is limited, blood glucose levels are maintained by the mobilisation of body reserves and the saline-infused ewes, although losing weight, had sufficient body reserves to maintain blood glucose concentrations. Therefore a normal pattern of LH release can probably be sustained as long as the animal can maintain normal blood glucose concentrations.

The significant changes in circulating levels of FSH over the course of this study are consistent with the response of gilts which, fed a dietary energy level twice that of control gilts, showed an increase in ovulation rate and a slower decline of FSH concentrations during luteolysis (Flowers *et al.* 1989). Interestingly, insulin concentrations in these gilts were elevated about 2 days before the changes in FSH were observed. In the present study, a very similar pattern of insulin and FSH concentrations was observed. Insulin concentrations would have increased immediately after the start of glucose infusion and after approximately 2·5 days of glucose infusion the plasma FSH concentrations declined in the glucose-infused ewes. These changes suggest an increased secretion of oestradiol and inhibin and may be due to an increase in the number of oestrogenic follicles. If this is the case it suggests that approximately 3 days of glucose infusion is sufficient to stimulate follicular development.

The rate of FSH decline during luteolysis is dependent on oestradiol and inhibin secreted from the follicles destined to ovulate (Baird *et al.* 1976). We suggest that the developing follicles in the glucose-infused ewes were exposed to higher levels of FSH for about 8 h longer in the period 20 to 28 h post-PG (140 to 148 h after the start of the insulin infusion; Fig. 4). Goerke & Dutt (1978) found that glucose infusion on day 16 of the oestrous cycle had no effect on ovulation rate but that it did alter the interval to oestrus. The slower rate of FSH decline following PG and the increased interval to the FSH surge in the glucose-infused ewes suggests that the production of pre-ovulatory oestradiol following luteolysis was delayed relative to the control animals. We have recently confirmed that glucose and insulin when infused directly into the ovarian artery of sheep with an ovarian autotransplant, can inhibit the secretion of oestradiol (Downing *et al.* 1993*b*, Downing 1994). The consequences of this suppressive effect of insulin may be to allow the selection of more than one ovulatory follicle leading to multiple ovulation during the follicular phase or to eventual increased oestradiol secretion during the luteal phase of the oestrous cycle.

Granulosa cells from immature rat ovaries have both a low mitogenic activity and a low ability to secrete oestradiol (Peluso *et al.* 1991). Exposure of these ovaries to FSH for 24 h increased the mitogenic activity of the granulosa cells without increasing steroidogenic capacity; a further 24 h of exposure to FSH increased the steroidogenic activity of the cells but at the expense of their mitogenic activity (Peluso *et al.* 1991). It is of interest to note that, once differentiated, these cells had an absolute requirement for FSH and that co-exposure to insulin increased their mitogenic activity and decreased their steroidogenic capacity (Peluso *et al.* 1991). In this system, insulin acts as a mitogen at the expense of differentiated functional activity. The elevated insulin that followed the infusion of glucose to ewes fed a low quality diet may allow follicles to grow for longer periods of time before terminal differentiation. Therefore an increase in insulin-mediated glucose uptake during the late luteal phase of the oestrous cycle may stimulate the growth of follicles and prevent them from undergoing atresia, thereby increasing the pool of ovulatory follicles.

There is considerable evidence to support this view. For example, when gilts on restricted energy intake were treated with insulin, there was a reduced level of atresia in follicles and the proportion of medium-sized follicles in the total follicle population was maintained (Matamoros *et al.* 1991). When cattle were fed increased energy they had

fewer follicle waves per oestrous cycle because the follicles in each wave grew larger and survived longer (Murphy *et al.* 1991). Insulin, infused into ewes during the late luteal phase of the oestrous cycle, increased ovulation rate (Hinch & Roelofs 1986) but did not do so in ewes given daily injections of insulin (Leury *et al.* 1990, Beam & Holcombe 1992). Insulin itself is not likely to be the stimulus for an increase in ovulation rate following an increase in glucose availability. Insulin responds to changes in nutrient availability and, when nutrients are in excess, insulin stimulates various activities in cells such as growth and increased metabolic activity. The increases in cellular functions occur only because of increased nutrient availability. Increased insulin levels alone are likely to have detrimental effects on tissues *in vivo* because they would induce hypoglycaemia.

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