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# Evidence for a Mammogenic Role of Growth Hormone in Ewes: Effects of Growth Hormone-Releasing Factor During Artificial Induction of Lactation<sup>1</sup>

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**ABSTRACT:** Thirty-two 1-yr-old nulliparous Prealpes du Sud ewes were randomly allocated in a 2 × 2 factorial design and induced to lactate by injection of estradiol (.5 mg·kg<sup>-1</sup>·d<sup>-1</sup>) and progesterone (1.25 mg·kg<sup>-1</sup>·d<sup>-1</sup>) for 7 d (d 1 to 7). On d 18, 19, and 20, ewes received 1 mg/kg of hydrocortisone acetate twice daily to induce lactogenesis. Experimental ewes (n = 16) received human growth hormone-releasing factor 1-29 NH<sub>2</sub> (hGRF 1-29 NH<sub>2</sub>) treatment (four daily × 100 μg hGRF i.v.) from d 10 to d 20. The other 16 ewes were controls. Half of both groups was maintained at either 8.5 h (ShD) or 15.5 h light (LD), and half of each subgroup was slaughtered on d 21. The remaining ewes were milked during a 6-wk period. Mammary gland epithelial tissue DNA concentration and liver growth hormone (GH) binding were evaluated on tissues from slaughtered ewes.

The estrogen-progesterone treatment induced mammary gland development and enhanced the plasma concentrations of prolactin (PRL), GH, and IGF-I between d 1 and 7; concentrations increased 1.5, 2.3, and 2.6 times, respectively ( $P = .002$ ). Between d 10 and 20, hGRF treatment enhanced ( $P < .001$ ) plasma concentrations of GH ( $5 \pm 1.4$  ng/mL on d 7 vs  $14.4 \pm 1.3$  ng/mL on d 20) and IGF-I ( $722 \pm 42$  ng/mL on d 7 vs  $1,281 \pm 82$  ng/mL on d 18). Mammary DNA concentration at d 21 was greater ( $P = .07$ ) for hGRF-treated ewes (1.2 vs .95 mg/g fresh tissue). Milk yield was greater ( $P < .025$ ) in the hGRF groups ( $246 \pm 25$  g/d vs  $128 \pm 40$  g/d). The long photoperiod regimen enhanced these responses. These results suggest that mammogenesis and/or early lactogenesis in ewes is in part controlled by GH.

Key Words: Somatotropin, Ewe Lactation, Mammary Glands

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## Introduction

Despite any clear scientific evidence, the conventional use of recombinant bovine somatotropin (**rbST**) during lactation to stimulate ruminant galactopoiesis is suspected by consumers of dairy products to result in hormonal residues and alters the image that milk is a "pure" source of proteins. To overcome such perceptions, we propose an alternative way to stimulate milk production. This requires a short period of hormonal stimulation of mammary growth by treatment with human growth hormone-releasing factor (**hGRF**; Kann et al., 1988) during the period of mammogenesis or early lactogenesis. The present

study was undertaken to further test this hypothesis. To avoid the confounding effects of fetal-placental hormones in pregnant ewes with exogenous hormones, the effects of hGRF (1-29 NH<sub>2</sub>) on mammogenesis and subsequent lactation were determined using nonpregnant ewes exposed to short- (**ShD**) or long-day (**LD**) photoperiods (Perier et al., 1986; Bocquier et al., 1990) and induced to lactate with exogenous steroids (Fulkerson et al., 1975; Head et al., 1980; Head et al., 1982).

## Materials and Methods

**Animals.** Thirty-two 1-yr-old nulliparous Prealpes du Sud ewes were allocated randomly in a 2 × 2 factorial arrangement. All ewes were induced to lactate. Sixteen ewes received hGRF and 16 served as controls under two different photoperiods (four subgroups of eight ewes each). From d 1 to 7, each ewe (mean weight 50 kg) received .5 mg/kg of estradiol-17β (**E<sub>2</sub>**) and 1.25 mg/kg of progesterone (**P<sub>4</sub>**)

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dissolved in ethanol in daily s.c. injections at 0900 and 1500. On d 18, 19, and 20, ewes received 1 mg/kg of hydrocortisone acetate twice daily to induce lactogenesis. All steroids were provided by Roussel-Uclaf (Romainville, France). At the end of the steroid treatment for induction of lactation (i.e., d 21), one-half of the ewes in each subgroup (four ewes/group) were slaughtered. From these ewes, the whole mammary glands, carefully dissected free of external fat to select mostly only the mammary parenchymal tissue, and liver samples (50 g) were individually stored at  $-80^{\circ}\text{C}$  until they were analyzed for DNA concentrations and growth hormone (GH) receptors. The remaining ewes were milked for 6 wk beginning on d 21. Milk yield was recorded daily by measuring weights of machine-collected milk at 0830 and 1530 and expressed as weekly means.

**Photoperiod Treatments.** Ewes were housed in two light-proof rooms for 6 wk before the beginning of steroid treatments and exposed to either long-day (LD, 15.5 h light) or short-day (ShD, 8.5 h light) conditions. Fluorescent lights provided 500 lx of intensity at 80 cm from the floor. Photoperiodic regimens were maintained for each group until slaughter or 6 wk of lactation.

**Synchronization of Estrous Cycles.** To eliminate effects of photoperiod on estrous cycles of ewes, which could also affect the response to steroids used to induce lactation, estrus was synchronized in all ewes using fluoroprogesterone vaginal sponges (40 mg) for 14 d and removed 8 d before the beginning of steroid treatment. Equine chorionic gonadotrophin (200 IU/ewe) was administered at the time vaginal sponges were removed. Both hormones were provided by Intervet (Angers, France).

**Human Growth Hormone-Releasing Factor Treatment.** From d 10 (3 d after the end of treatment with estradiol and progesterone) to d 20, one-half of the ewes exposed to LD or ShD received four daily i.v. injections of 100  $\mu\text{g}$  of hGRF (1–29  $\text{NH}_2$ ) provided by Sanofi Recherche (Montpellier, France) in 1 mL of saline at 0800, 1030, 1300, and 1530. Control ewes received i.v. injections of 1 mL of saline at the same times.

**Blood Sampling.** Blood sampling was accomplished with evacuated tubes containing EDTA  $\text{Na}_2$  (6 mg/5 mL) on d  $-42$ , 0, 7, 11, 13, 15, 16, 18, and 20 at 1100 and 1330 to follow the general release profiles of prolactin (PRL), GH, and IGF-I in plasma. For more precise determinations of GH after hGRF injections, serial samples were collected on d 10 and 17 from all hGRF-treated ewes from 10 min before administration of hGRF and every 10 min thereafter until the next injection of hGRF. Plasma was collected and stored at  $-20^{\circ}\text{C}$  until it was used for RIA.

**Radioimmunoassays.** All assays were double antibody procedures. Iodination of hormones was performed with an adaptation of the chloramine T method (Kann, 1971) with  $[^{125}\text{I}]\text{Na}$  (IMS 300,

Amersham, Buckinghamshire, U.K.). Labeled hormones were purified by exclusion chromatography using an Ultrogel AcA-5/4 (IBF, Villeneuve la Garenne, France) column ( $.9 \times 30$  cm) equilibrated and eluted with appropriate buffer. Fractions (.5 mL) were collected, and those containing the peptide were stored in aliquots containing glycerol (60% vol/vol) at  $-30^{\circ}\text{C}$  until used. Standard curves were developed in hormone-free plasma aliquots equivalent to those for plasma samples assayed for PRL and GH.

For IGF-I assays, plasma samples were subjected to acid ethanol extraction (Daughaday et al., 1980) to separate IGF-I from IGF binding proteins with the cryoprecipitation step suggested by Breier et al. (1991). Recovery with this extraction procedure reduced variation among samples ( $92 \pm 4$  %, mean  $\pm$  SEM,  $n = 12$ ) when 800 cpm of  $[^{125}\text{I}]\text{IGF-I}$  was added before extraction. Results of the RIA were corrected for efficiency of recovery during extraction. Recombinant human IGF-I (rh metIGF-I, batch 742.44), a gift from D. Burleigh (International Mineral & Chemical Corp., Terre Haute, IN), or recombinant bIGF-I (batch 5101), a gift from R. Collier (Monsanto, St. Louis MO), were used as standards and for iodination; similar results were achieved using the two sources. Rabbit antiserum UB 3189 was a gift from L. Underwood and J. J. Van Wyk (University of North Carolina, Chapel Hill) through the National Hormone and Pituitary Program of the NIADDK (Baltimore, MD). Buffer for the RIA and eluent for column chromatography after iodination was a phosphate-protamine buffer, pH 7.5, containing Tween 20 (.05%). Protamine was substituted for BSA, which is often contaminated with IGF-I or IGF-IBP. Addition of IGF-I to ovine plasma before extraction (50 ng/mL up to 1  $\mu\text{g}/\text{mL}$ ) was quantitatively recovered for the entire range of added material ( $90.3\% \pm .8$ , mean  $\pm$  SEM,  $n = 19$ ), giving validation for an estimation of total IGF-I in ovine plasma. This nonequilibrium RIA had a sensitivity of 4.38 ng/mL. The IGF-I was measured only in plasma samples from d  $-42$ , 0, 7, 16, and 18. The intraassay CV was 8.3%.

The PRL RIA has been described (Kann, 1971). The PRL PS7, a gift from NIH (24.8 IU/mg), was used as the tracer hormone and standard. This RIA had a sensitivity of .0625 ng/mL (PRL PS7). The intra- and interassay CV were 5.3% and 6.0%, respectively.

The GH RIA used anti-ovine GH antiserum provided by the NIADDK (AFP C0123080). The oGH I-4 (1.4 IU/mg), a gift of the NIADDK, was used for labeling and as standard. The GH assay had a sensitivity of .625 ng/mL (oGHI-4, 1.7 IU/mg). The intra- and interassay CV were 8.4 and 6.8%, respectively.

**Liver Growth Hormone Receptors.** Liver cell microsomes were prepared from each sample of liver and stored until used at  $-20^{\circ}\text{C}$  in 25 mM Tris HCl, pH 7.5, .02% sodium azide, 1 mM phenylmethylsulfonyl fluoride, and 10 mM  $\text{MgCl}_2$  buffer. After assay for protein

content (Lowry et al., 1951), .3 mg of membranes was incubated with 80,000 cpm of [ $^{125}$ I]GH for 18 to 20 h at room temperature. An excess of unlabeled GH added (1  $\mu$ g/tube) almost completely reversed the binding of [ $^{125}$ I]GH and was considered to represent nonspecific binding. Specific binding was calculated as total binding minus nonspecific binding and expressed as a percentage of radioactivity added per tube.

**DNA Concentration of the Secretory Epithelium of Mammary Glands.** One-half of the mammary gland epithelial tissue stored at  $-80^{\circ}\text{C}$  was mechanically ground and homogenized in ice-cold .7 M guanidine chloride, .1 M sodium acetate, pH 7.5, containing 1% mercaptoethanol. The homogenate was centrifuged at  $8,000 \times g$  for 30 min, the volume of the resulting supernatant was estimated, and aliquots (V1) were precipitated overnight with  $3 \times V1$  of 100% ethanol. Insoluble material containing nucleic acids and most proteins was sedimented by centrifugation at  $30,000 \times g$  for 30 min. After discarding the supernatant, the pellet was resuspended in sterile 2 M ammonium acetate (volume equivalent to V1) and precipitated again overnight in two volumes of 100% ethanol. After a 30-min centrifugation at  $30,000 \times g$ , the supernatant containing guanidine chloride, nucleotides, and some proteins was discarded, and the insoluble pellet was resuspended in 100 mM Tris HCl, pH 9, .5% SDS (volume V1). Total nucleic acids were then extracted in one volume (V1) of phenol and  $.1 \times V1$  of 2 M sodium acetate, pH 5, and  $2 \times V1$  of 100% ethanol. Insoluble nucleic acids were recovered following centrifugation for 30 min at  $30,000 \times g$ . The resulting pellet was resuspended in 10 mM Tris HCl, pH 7.5, .1% SDS. Absorbance at 260 nm (A<sub>260</sub>) of this solution was evaluated on the basis  $20 \times A_{260} = 1$  mg of DNA. This method was developed in the laboratory at the time of the experiments reported here, was reliable for quantitative measurement of DNA extracted in an undegraded form from mammary glands of different species at various stages of development, and has been recently validated after confrontation with a simpler and less time-consuming technique (fluorescence of DNA after binding to bisbenzimidazole) according to Labarca and Paigen (1980).

**Statistical Analysis.** Different models were run when possible for each variable and included two-way analysis of variance with or without covariables (hormone concentrations before and/or after treatments, photoperiod regimen). Comparisons were made with ANOVA procedures appropriate for the split-plot design using the GENSTAT 4.04 statistical package (Numerical Algorithms Group, Oxford, U.K.) for analysis of variance to evaluate time or time  $\times$  treatments effects with introduction of polynomial terms. Residual error sum of squares was used to calculate the SEM and to detect significant differences between control and treatments.

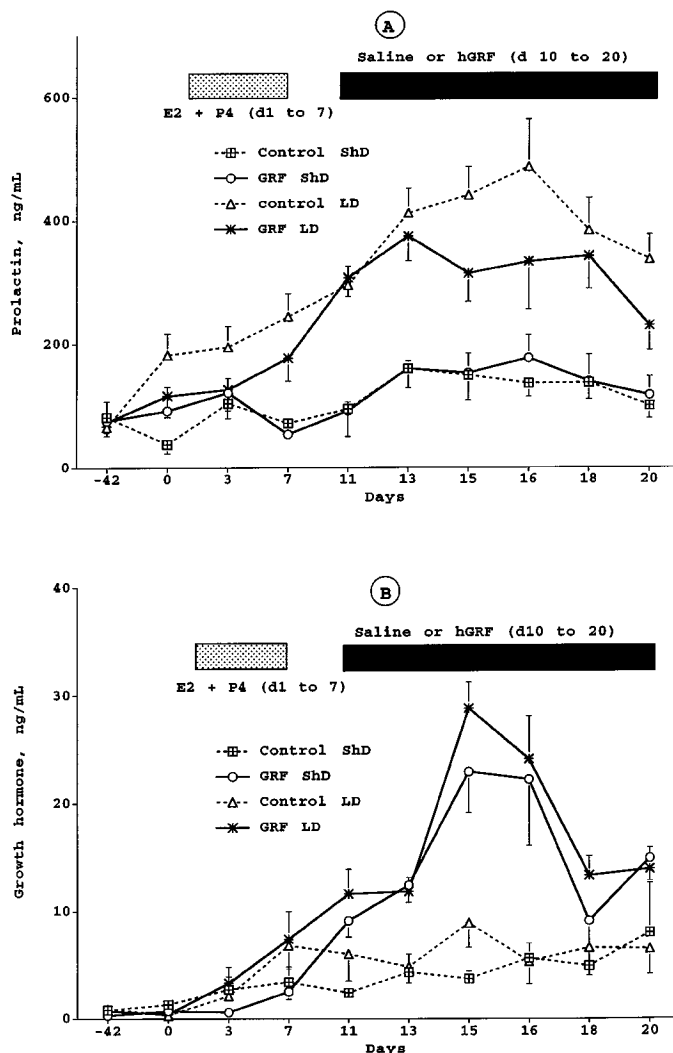


Figure 1. Mean plasma concentration ( $\pm$  SEM) of (A) ovine prolactin and (B) ovine growth hormone during the artificial induction of lactation with estradiol-progesterone (d 1 to 7) and hydrocortisone (d 18 to 20) treatments, relative to different photoperiod (short day = ShD; long day = LD) and human growth hormone-releasing factor (hGRF) treatment.

## Results

### Hormones

If not specifically mentioned, each daily hormone value is the mean of values measured in plasma samples taken at 1100 and 1330 (i.e., 30 min after the injections of hGRF).

**Prolactin.** From the day before exposure to the different photoperiods (i.e., d  $-42$  to the beginning of steroid treatment on d 0), PRL increased ( $P < .003$ ) from  $70 \pm 16$  to  $149 \pm 20$  ng/mL in LD ewes (Figure 1A), but values did not increase during those same days for ShD ewes ( $79 \pm 10$  vs  $65 \pm 21$  ng/mL). Although lower in ShD than in LD ewes, PRL increased from d 0 to 16 and then declined to d 20 (see

Figure 2A). The hGRF treatment had no effect on PRL levels in ewes.

**Growth Hormone.** Before steroid treatment, GH was similar among the four groups of ewes (Figure 1B and Figure 2). In response to the estrogen-progesterone treatment, GH increased from basal levels to  $2.2 \pm .5$  ng/mL on d 3 and to  $5.1 \pm 1.9$  ng/mL on d 7 ( $P = .002$ ).

There was a trend for GH levels to be higher for ewes exposed to LD ( $7.1 \pm 1.7$  ng/mL) than for those exposed to ShD ( $3 \pm .8$  ng/mL) on d 7. Between d 10 and 20 in ewes that received no hGRF, GH levels were relatively stable. However, basal levels of GH increased in ewes subjected to hGRF after d 10. Although slightly higher in ewes exposed to LD

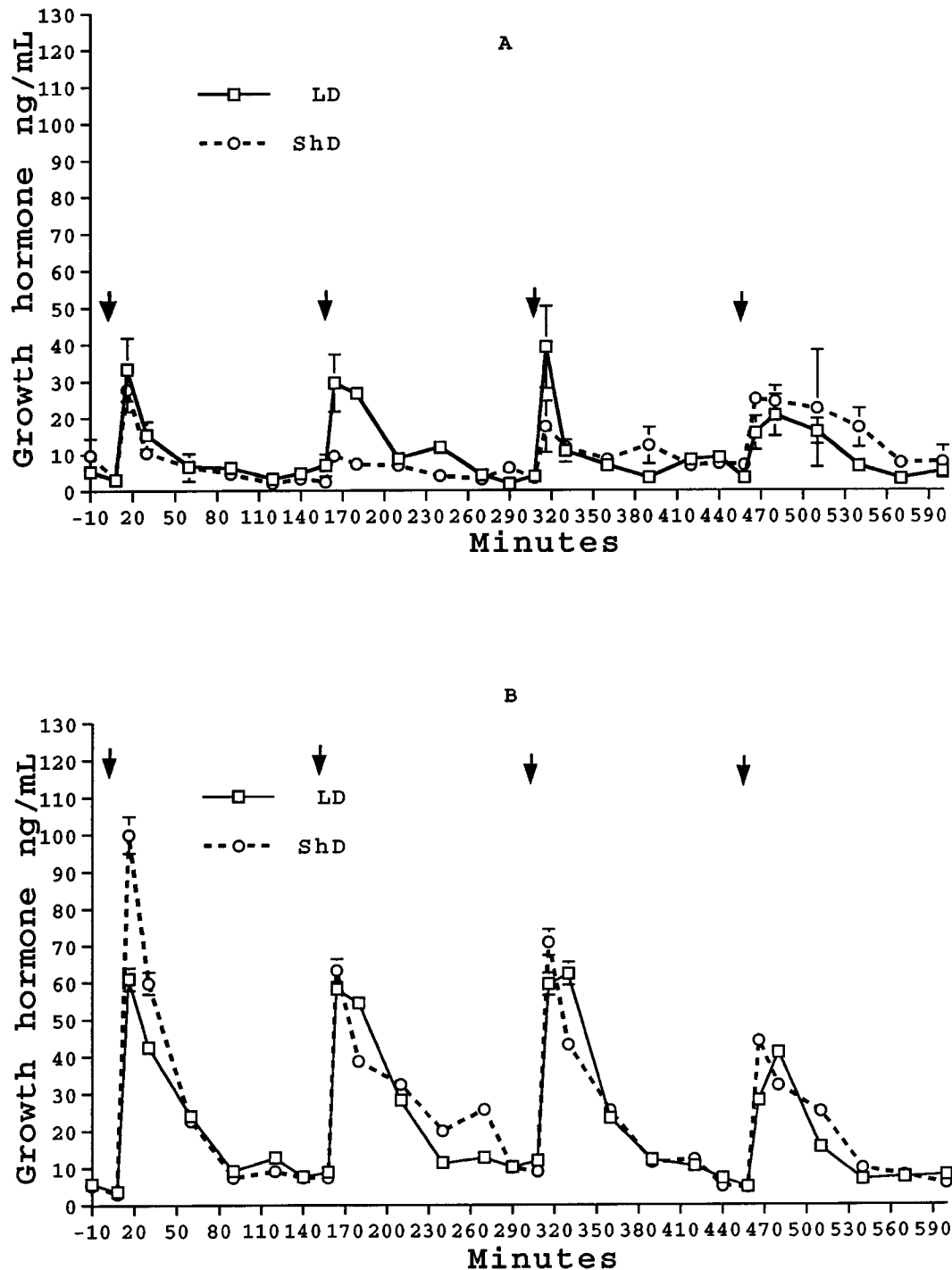


Figure 2. Mean growth hormone response ( $\pm$  SEM) of eight ewes on d 10 (A, 1st d of human growth hormone-releasing factor injections) or on d 17 (B) to four daily i.v. 100- $\mu$ g injection of human growth hormone-releasing factor (1-29 NH<sub>2</sub>). Ewes received either 15.5 h of light/d (LD; —; n = 4) or 8.5 h of light/d (ShD; - - - ; n = 4). Injections were given at times shown by vertical arrows.

Table 1. Changes in plasma IGF-I (mean  $\pm$  SEM) relative to short-day (ShD; 8.5 hours) or long-day (LD; 15.5 hours) photoperiods or to human growth hormone-releasing factor (hGRF) treatment irrespective of photoperiod regimen

Day	ShD (n = 16)	LD (n = 16)	Control (n = 16)	hGRF (n = 16)	ALL (n = 32)
-42	250 $\pm$ 14 <sup>a</sup>	242 $\pm$ 21 <sup>a</sup>	248 $\pm$ 1 <sup>a</sup>	252 $\pm$ 24 <sup>a</sup>	250 $\pm$ 14 <sup>a</sup>
0	248 $\pm$ 20 <sup>a</sup>	313 $\pm$ 25 <sup>b</sup>	282 $\pm$ 20 <sup>b</sup>	280 $\pm$ 28 <sup>b</sup>	281 $\pm$ 17 <sup>b</sup>
7	613 $\pm$ 43 <sup>c</sup>	831 $\pm$ 62 <sup>c</sup>	681 $\pm$ 50 <sup>c</sup>	763 $\pm$ 67 <sup>c</sup>	722 $\pm$ 42 <sup>c</sup>
16	995 $\pm$ 61 <sup>d</sup>	1,241 $\pm$ 94 <sup>e</sup>	886 $\pm$ 44 <sup>e</sup>	1,351 $\pm$ 73 <sup>e</sup>	1,110 $\pm$ 59 <sup>e</sup>
18	1,191 $\pm$ 117 <sup>d</sup>	1,370 $\pm$ 114 <sup>e</sup>	1,048 $\pm$ 66 <sup>d</sup>	1,514 $\pm$ 127 <sup>e</sup>	1,281 $\pm$ 82 <sup>e</sup>

a,b,c,d,e Means in the same row or column without a common superscript differ ( $P < .001$ ).

compared to ShD, the difference was not significant. Each i.v. injection of hGRF was followed by an increase in GH on d 10, and GH responses were higher ( $P = .0017$ ) on d 17 than on d 10. On d 10 and 17, analysis of the GH responses as area under the curves at 0800, 1030, 1300, and 1530 revealed no interaction between photoperiod regimen and hGRF treatment (Figure 2).

**Insulin-Like Growth Factor I.** At the beginning of the photoperiodic treatment on d -42, plasma concentrations of IGF-I averaged  $250 \pm 14$  ng/mL (n = 32). Concentrations of IGF-I remained unchanged on d 0 for ShD ewes ( $248 \pm 20$  ng/mL, n = 16), but they increased ( $P < .001$ ) for LD ewes ( $313 \pm 25$  ng/mL; Table 1). Steroid administration increased plasma concentrations of IGF-I in all ewes ( $P < .001$ ), regardless of the photoperiodic treatment ( $282 \pm 20$  ng/mL on d 0 and  $681 \pm 50$  ng/mL on d 7).

The effect of the hGRF injection on IGF-I was also significant ( $P < .001$ ), irrespective of photoperiod regimen; concentrations were  $763 \pm 67$  ng/mL before hGRF treatment on d 7,  $1,351 \pm 73$  ng/mL on d 16, and  $1,514 \pm 127$  ng/mL on d 18 (Table 1). Ewes exposed to LD had higher plasma IGF-I values than those exposed to ShD (Table 2).

**Growth Hormone Hepatic Receptors.** There was a stimulatory ( $P = .01$ ) effect of the photoperiod regimen across hormone treatments on specific binding of [<sup>125</sup>I]GH by hepatic microsomes ( $10.7 \pm .8\%$  for LD vs  $8.4 \pm .4\%$  for ShD ewes). The hGRF treatment stimulated ( $P = .04$ ) binding of GH to hepatic membranes ( $10.4 \pm .5\%$  for treated ewes vs  $8.7 \pm .4\%$  for control ewes). The interaction between photoperiod and hGRF treatment was not significant ( $P = .21$ ). Nonlinear regression analysis of the competitive binding data using GH as labeled and unlabeled ligands detected no significant variation in the association constant of GH to its receptors due to either photoperiod or hGRF treatment ( $K_a = 3.2 \pm .2$  L/nM). However, hGRF treatment affected the concentration of GH receptors on hepatic membranes ( $84 \pm .6$  fM/mg protein for hGRF-treated vs  $63.5 \pm 5$  fM/mg protein for control ewes,  $P = .06$ ). The interaction between photoperiod and treatment was not significant ( $P = .35$ ).

#### DNA Concentration of the Mammary Epithelium.

The overall effect of hGRF on the mammary gland epithelial DNA concentration tended to be significant irrespective of photoperiod regimen ( $P = .07$ ) when compared to untreated ewes. A similar analysis that accounted for the photoperiod regimen showed that ShD-hGRF ewes had higher ( $P = .02$ ) mammary gland epithelial DNA concentration (Figure 3) than LD-hGRF ewes. The main effect of photoperiod and the photoperiod  $\times$  hGRF interaction were not significant.

**Milk Yield.** Two variables were studied: the mean milk yield during each week during the 6-wk experimental period, with a particular analysis on the 1st wk of lactation, and the slopes of the mean lactation curves. The increase in milk yields was linear in all groups of ewes ( $P < .001$ , Figure 4). The overall milk production during the 6 wk was significantly higher for hGRF-treated ewes than for controls regardless of the photoperiod ( $P = .025$ ), and this effect was detected as early as the 1st wk of lactation according to the correlation between the slope of the lactation curve of hGRF-treated ewes and the milk production ( $R = .7$ ,  $P = .05$ ). A similar correlation was not observed for control ewes ( $R = .22$ ,  $P = .6$ ). The LD induced significantly higher milk yields than ShD during the 1st wk of lactation regardless of the hGRF treatment (61% higher,  $P = .06$ ), but this effect

Table 2. Changes in plasma IGF-I (mean  $\pm$  SEM) relative to the human growth hormone-releasing factor (hGRF) treatment for short-day (ShD; 8.5 hours) or long-day (LD; 15.5 hours) photoperiod regimens

Day	Control ShD (n = 8)	hGRF ShD (n = 8)	Control LD (n = 8)	GRF LD (n = 8)
-42	258 $\pm$ 27 <sup>a</sup>	256 $\pm$ 30 <sup>a</sup>	238 $\pm$ 19 <sup>a</sup>	247 $\pm$ 40 <sup>a</sup>
0	255 $\pm$ 22 <sup>a</sup>	241 $\pm$ 34 <sup>a</sup>	308 $\pm$ 31 <sup>b</sup>	318 $\pm$ 40 <sup>b</sup>
7	579 $\pm$ 64 <sup>c</sup>	648 $\pm$ 59 <sup>d</sup>	783 $\pm$ 60 <sup>d</sup>	879 $\pm$ 110 <sup>d</sup>
16	828 $\pm$ 53 <sup>e</sup>	1,162 $\pm$ 71 <sup>f</sup>	943 $\pm$ 68 <sup>e</sup>	1,539 $\pm$ 86 <sup>g</sup>
18	1,020 $\pm$ 121 <sup>e</sup>	1,363 $\pm$ 188 <sup>g</sup>	1,075 $\pm$ 63 <sup>f</sup>	1,665 $\pm$ 164 <sup>g</sup>

a,b,c,d,e,f,g Means in the same row or column without a common superscript differ ( $P < .001$ ).

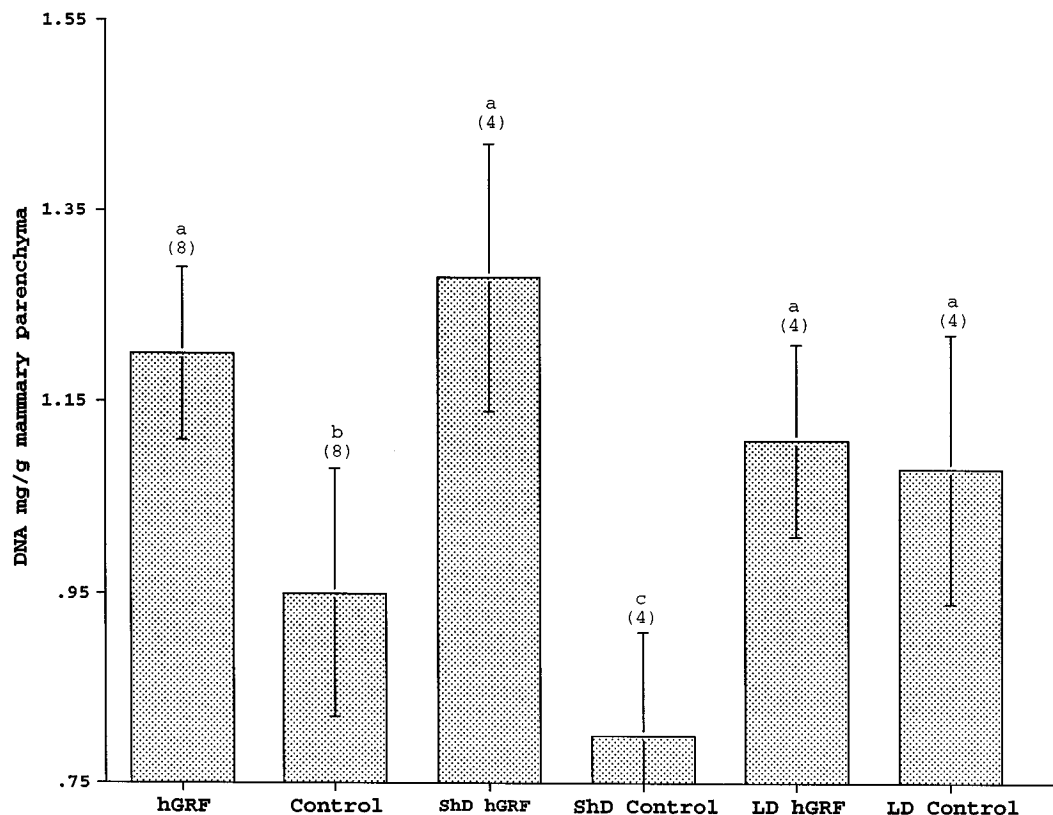


Figure 3. Mean DNA concentrations ( $\pm$  SEM) measured in mammary glands of the four groups of slaughtered ewes at the end of the period of induction of lactation (d 21); number of ewes is in parentheses. Bars with different superscripts differ ( $P < .001$ ). hGRF = human growth hormone-releasing factor; ShD = short-day treatment (8.5 h of light/d); LD = long-day treatment (15.5 h of light/d).

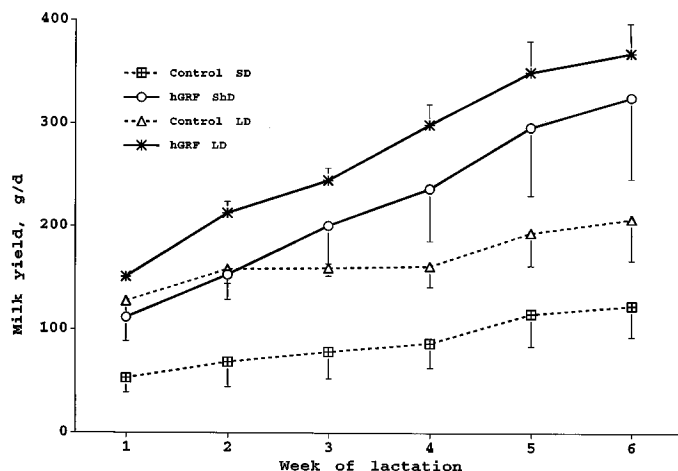


Figure 4. Mean daily milk yield ( $\pm$  SEM) during the first 6 wk after the period of artificial lactation induction for the four groups of milked ewes. The human growth hormone-releasing factor (hGRF) treatment increased ( $P < .025$ ) milk yield during the 6-wk period, and the LD photoperiod increased ( $P < .06$ ) milk yield during the 1st wk of lactation. ShD = short-day treatment (8.5 h of light/d); LD = long-day treatment (15.5 h of light/d).

became less evident during the next 5 wk (27% higher for the total milk production of 6 wk,  $P = .26$ ). The enhancement of the total milk yield of hGRF-treated ewes was greater for the ShD group (148% more,  $P = .02$ ) than for the LD group (31% more,  $P = .31$ ). The LD induced a significant effect during the 1st wk of milk production when compared to ShD for the untreated group ( $P = .018$ ), and this effect disappeared with the hGRF group ( $P = .37$ ).

## Discussion

Results from the present experiment suggest the important role of endogenous GH in improving mammary development or early lactogenesis in ewes artificially induced to lactate, particularly when they were maintained under a ShD photoperiod (i.e., when prolactin levels were lower than under LD conditions).

Ewes that received four daily injections of hGRF responded at each i.v. injection with an increased secretion of GH. Values recorded for GH were comparable to those described earlier for goats, ewes, and cows under similar stimulation (Hodate et al., 1984; McCutcheon et al., 1984; Enright et al., 1985;

Hart et al., 1985; Lapierre et al., 1985; Kann et al., 1988). On d 17, at the end of the treatment period, the areas under the GH curves were greater ( $P = .0007$ ) than at the beginning (d 10, Figure 2). Similar observations were made in other experiments in which 1.5 mg of hGRF was administered s.c. to pregnant ewes twice daily either from d 105 to 115 or from d 136 to 146 of gestation (Kann et al., 1988, 1989) and were comparable to the findings of Moseley et al. (1987) after continuous i.v. infusion of hGRF to young bull calves during 20 d. This variation of hypophysis sensitivity could be connected either to a progressive autoinduction of hGRF receptors at the pituitary level or to a higher capacity of the pituitary to synthesize and/or release more GH after daily hGRF treatments. However, it is clear that, as previously reported from in vitro and in vivo data from sheep and humans (Giustina et al., 1994; Sartin et al., 1994), administration of hydrocortisone apparently inhibits GH response to hGRF on the last day of hGRF treatment (Figure 1B).

The magnitude of the increase in milk production was enhanced in LD ewes. In addition to the enhancement of prolactin already described (Ravault and Ortavant, 1977; Bocquier et al., 1990), our results indicate that lengthening photoperiod also increased circulating plasma concentrations of GH and IGF-I and hepatic concentrations of GH receptors. The finding that LD stimulated GH plasma levels, liver GH receptors, and plasma IGF-I suggests modulation of the GH receptor, possibly through an up-regulation mechanism in which GH may induce its own hepatic receptor (GHR), as suggested by Sauerwein et al. (1991).

The circulating levels of IGF-I were parallel to the GH rise in LD ewes (Figure 4 and Table 2). This observation is consistent with higher numbers of GH liver receptors in LD ewes.

In ShD ewes, the effect of hGRF treatment clearly induced an enhancement of mammogenesis as measured through the mammary gland epithelial DNA concentration when compared to that of control ShD ewes. When milk yields were analyzed, the increase in milk production was associated with highest DNA concentrations in mammary epithelial tissues.

Moreover, the steroid treatment by itself, which was known to stimulate PRL (Fulkerson et al., 1975; Head et al., 1982) and GH (Hooley et al., 1981), had a similar yet undescribed triggering effect on IGF-I levels. This could be anticipated from studies that demonstrated the role of steroids in IGF-I secretion (Wilson et al., 1984; Ho et al., 1987; Breier et al., 1988; Adesianya et al., 1996). The IGF-I receptor has been found in ewe (Diesenhaus et al., 1988) and bovine mammary tissue (Campbell and Baumrucker, 1986; Dehoff et al., 1988), and in vitro studies have shown that IGF-I stimulated DNA synthesis (mammogenesis) in ovine or bovine mammary epithelial

cells (Winder et al., 1989; McGrath et al., 1991) and in explants of bovine mammary tissue (Baumrucker and Stemberger, 1989). However, IGF-I has been shown to stimulate lactose production (lactogenesis) in mammary acini cultures (Baumrucker, 1986). Therefore, it can be suggested that in artificial induction of lactation, stimulation of mammary gland epithelial DNA concentrations and/or early lactogenesis are in part related to the enhancement of GH and IGF-I plasma concentrations together with the recorded GHR increase. It has been suggested, according to the somatomedin hypothesis formulated by Daughaday et al. (1972), that mammary effects of GH during galactopoiesis could be partially mediated through the increase of circulating IGF-I levels recorded with the enhancement of milk performance of either rbST-treated lactating cows (Peel et al., 1985; Cohick et al., 1987; Davis et al., 1987; Hadsell et al., 1988) or hGRF-treated lactating ewes or cows (Kann, unpublished results). The main difference between these manipulations of galactopoiesis through GH administration or enhancement and the results of artificial induction of lactation is that enhancement of milk yields during galactopoiesis lasted only during the duration of the hormone stimulation; milk yields returned to pretreatment values within a few days, but a 10-d stimulation in artificial induction of lactation improved milk yield for at least 6 wk, indicating a long-lasting effect even after the hormone treatment ended. Therefore, even if we cannot exclude an effect of hGRF through GH and IGF-I on early lactogenesis, a main effect on mammogenesis seems more likely in accordance with the observed increase of mammary parenchyma DNA concentrations occurring before any milking stimulus with a concomitant sustained enhanced milk yield.

In conclusion, results of this study indicate that GH can significantly enhance mammary growth and differentiation following artificial induction of lactation, and the increase in GH is associated with higher milk yield for ewes. One may speculate on the physiological relevance of these findings because levels of GH recorded in the present study are never found during the normal life of ewes. Ovine chorionic somatotropin (**oCS**) is secreted at much higher levels near the end of gestation (Chan, 1978) and belongs to the PRL-GH family (Colosi et al., 1989). The oCS interacts with either GHR (Breier et al., 1994) or with its own receptors on liver cells (Freemark et al., 1987) and may be an important component of the pluri-hormonal complex responsible for mammogenesis (Schams et al., 1984; Forsyth, 1986). It may be suggested, therefore, that the ability of GH to trigger mammogenesis and/or early lactogenesis, which has already been described during late pregnancy (Kann et al., 1988) at a period when mammogenesis takes place in ewes (Denamur, 1966), reflects the sensitivity of the ovine mammary gland to hormones of the somatotrophic axis, such as oCS.

## Implications

Stimulation of growth hormone by human growth hormone-releasing factor during a 10-d period at the end of a treatment to induce lactation in ewes enhanced milk production. This enhancement of milk yield despite the cessation of the growth hormone increase contrasts with the well-known temporal effect of growth hormone during galactopoiesis on milk yield and may indicate that growth hormone has an important role in mammogenesis and(or) lactogenesis in ewes.

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