

## Characteristics of growth hormone response to the administration of growth hormone-releasing hormone (GRF) in the lamb

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**Summary.** Human growth hormone releasing hormone (GRF 1-44 or GRF 1-29) was administered to lambs at two different physiological stages (suckling : 5-6 week-old and weaned : 14-15 week-old) when growth hormone (GH) secretory patterns were different : suckling lambs exhibited flat basal GH profiles (5-10 ng/ml) while the weaned lambs had frequent spontaneous episodes of GH release (15-65 ng/ml).

The *iv* injection of GRF evoked an immediate release of GH. In each case, plasma GH levels reached a maximum 1-4 min after the injection. The secretory spike was multiphasic and lasted 30-60 min. Administration of GRF (0.1 µg/kg) in weaned lambs induced GH pulses with an amplitude comparable to that of endogenous peaks. The induction of a GH peak occurred even when a spontaneous peak immediately preceded the GRF injection. Also, spontaneous peaks were observed during the hour following a GRF-induced GH peak.

In sucking lambs, GRF injected intravenously as a single bolus in a range of 0.01 to 05. µg/kg (2 to 100 pmoles/kg) stimulated GH release in a dose-dependent manner.

Chronic administration of GRF (0.75 nmole GRF 1-44 or GRF 1-29 per kg twice daily for 21 days) in newborn lambs increased significantly ( $p < 0.001$ ) the acute response to GRF during the course of the treatment. GH response to GRF 1-44 and GRF 1-29 was the same.

These data show that lambs are highly responsive to GRF action during both suckling and weaning and suggest that there is no *in vivo* desensitization of the pituitary gland after acute or chronic GRF administration.

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## Introduction.

Since human growth hormone-releasing hormone (hGRF) was characterized and synthesized (Guillemin *et al.*, 1982 ; Rivier *et al.*, 1982), extensive *in vivo* and *in vitro* studies have been initiated (Guillemin *et al.*, 1984). Its specificity with respect to growth hormone (GH) secretion is well documented (Wehrenberg *et al.*, 1982 ; Thorner *et al.*, 1983 ; Moseley *et al.*, 1984). Although the amino acid sequence of GRF is species-specific (Ling *et al.*, 1984), GRF of human origin has been demonstrated to be extremely potent in a number of very different species such as rats (Rivier *et al.*, 1982 ; Wehrenberg *et al.*, 1982), ruminants (Hodate *et*

al., 1984), pigs (Kraft *et al.*, 1985) and fowl (Scanes *et al.*, 1984). In sheep, hGRF has been shown to stimulate GH secretion in the fetus (Ohmura *et al.*, 1984), as well as in cultured pituitary cells (Law *et al.*, 1984; Blanchard *et al.*, 1987). Therefore hGRF appears to be an appropriate form of the peptide to use to investigate dynamic changes of GH release in sheep.

Plasma GH levels are very high in the ovine fetus at late gestation, then fall abruptly at birth (Bassett and Alexander, 1971; Gluckman *et al.*, 1979a). However, there is no decrease in absolute or relative pituitary GH content during this period (Charrier, 1973). The infusion of somatostatin into fetal or adult sheep causes, at best, 50% inhibition of GH release (Davis and Anfinson, 1975; Gluckman *et al.*, 1979b). Thus it is likely that another mechanism is involved in the postnatal decrease in GH secretion, either a decrease in pituitary sensitivity to GRF action or a decreased stimulation of this gland.

The present series of experiments were designed to study the *in vivo* responsiveness of lambs to GRF. We have also examined for possible desensitization of the pituitary gland in lambs receiving a single dose of GRF or chronic treatment.

### Materials and methods.

All the animals used in this study were Mérinos d'Arles lambs. They were kept inside with their mother until weaning at 7 week of age and under natural conditions of light and temperature. They received food and water *ad libitum*. The lambs were conditioned to experimental manipulations from the time of birth.

When the blood sampling intervals were every 5 min or longer, the blood was taken by venipuncture *into* heparinized glass tubes and GRF was injected intravenously. With shorter intervals of time, the blood was collected *into* heparinized glass tubes through an indwelling catheter placed *into* the jugular vein two days before. In this case, GRF was injected through a second catheter followed by 1 ml of sterile normal sheep serum (NSS).

For all the acute tests, GRF was administered intravenously (iv). The test time varied except for the chronic treatment regime. During this regime, subcutaneous (sc) injections of GRF were given at 8 am and 8 pm for 21 days and blood samples were drawn beginning at 30 min prior to the 8 am injection.

Human GRF 1-44 (SR95228) and GRF 1-29 (SR95515) were gifts of Sanofi-Recherche (Montpellier). The powder was dissolved in sterile saline prior to use. Just before injection, 0.5 ml of the solution was diluted with 0.5 ml of NSS. The vehicle consisted of 1 ml of sterile saline/NSS (1 : 1).

Plasma was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  until assayed. Ovine GH (oGH) was measured by specific radioimmunoassay using a double antibody separation method. Reagents for the oGH assay were supplied by the National Hormone and Pituitary Program (NIADDK, Bethesda), except for the second antibody (sheep anti-rabbit-gammaglobulin serum) which was prepared in our lab. The sensitivity of the assay was 0.5 ng/ml and the intra-assay variation was 5%. All samples from the same experiment were run in the same assay at the appropriate dilution.

## Results.

Figure 1 shows the typical effect of GRF 1-44 ( $2 \mu\text{g}/\text{kg}$ ) injected as a bolus in a representative 15-week old lamb. Plasma GH levels rose during the first min and were maximum ( $130 \text{ ng}/\text{ml}$  in this case) 1-4 min after the injection of GRF. Secondary peaks of GH were observed during the 30 min following the injection after which plasma GH returned to basal levels. The multiphasic nature of the GH peak cannot be seen and the highest plasma GH levels were not measured when samples were taken every 10 min (fig. 1).

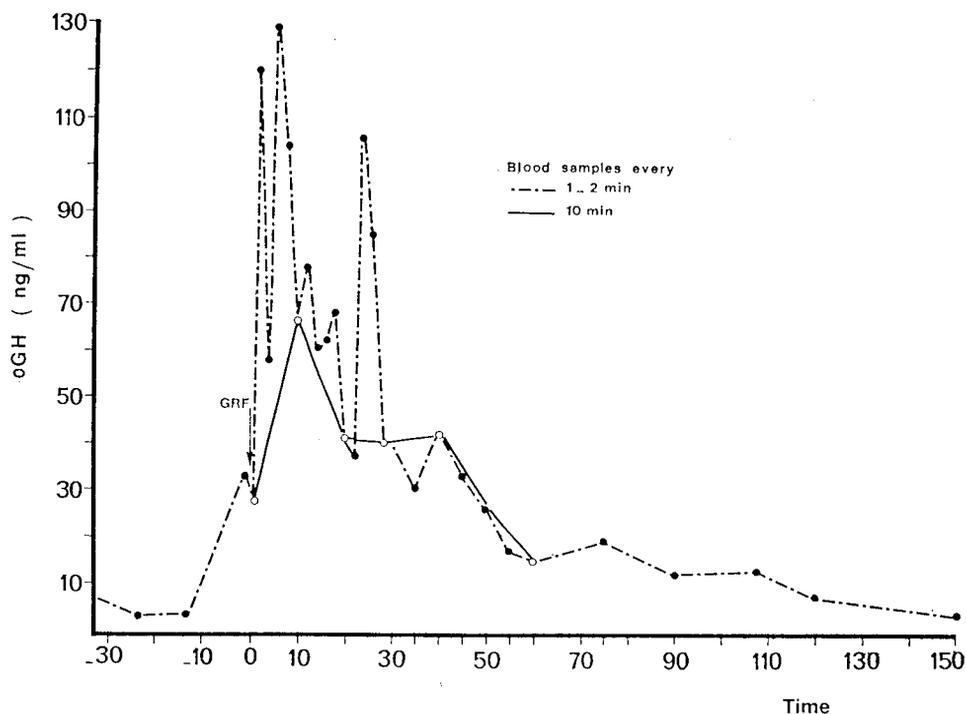


FIG. 1. — Kinetics of plasma GH levels in a 15-week old lamb given a single iv bolus of GRF 1-44 ( $2 \mu\text{g}/\text{kg}$ ). The arrow indicates when GRF was administered. Representation of the same GH secretory profile but using 1-2 min (---) or 10 min (—) blood sampling intervals.

Figure 2 shows the GH response to GRF 1-44 ( $0.1 \mu\text{g}/\text{kg}$ ) of four weaned lambs injected randomly at different times during their normal endogenous patterns of GH secretion. Using this lower dose, the amplitude of the GH peak subsequent to GRF injection was similar to spontaneous peaks and was only biphasic: the first occurred 3-9 min and the second 9-15 min after the injection of GRF, the two peaks being separated by 6-9 min. Plasma GH levels returned to basal concentrations within 30 min (fig. 2 b, c) except when a spontaneous peak of GH followed the GRF-induced one (fig. 2a). The lambs were responsive to GRF even when a spontaneous peak of GH had occurred just prior to (fig. 2c) or during

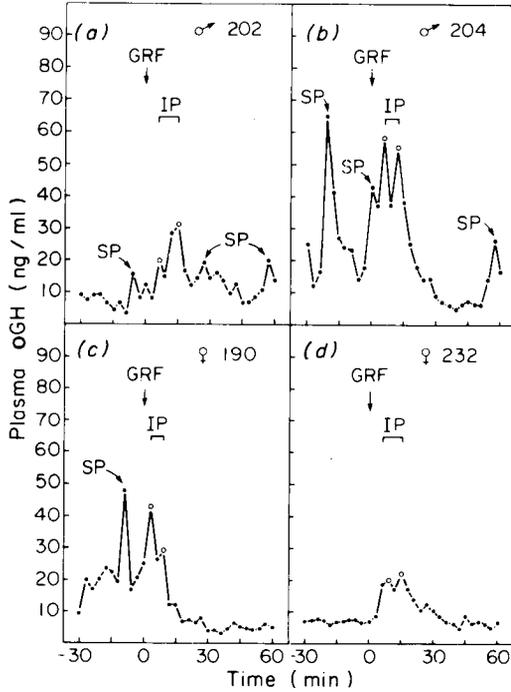


FIG. 2. — GH response in male and female weaned lambs (14-15 week old) given a single iv bolus of GRF 1-44 (0.1  $\mu\text{g}/\text{kg}$ ). Blood was sampled every 3 min. The animals depicted were chosen because they best represented various endogenous GH secretory patterns, the timing when GRF was injected and responsiveness to GRF. Arrows indicate GRF injections at zero time. SP : spontaneous peak ; IP : GRF-induced peak.

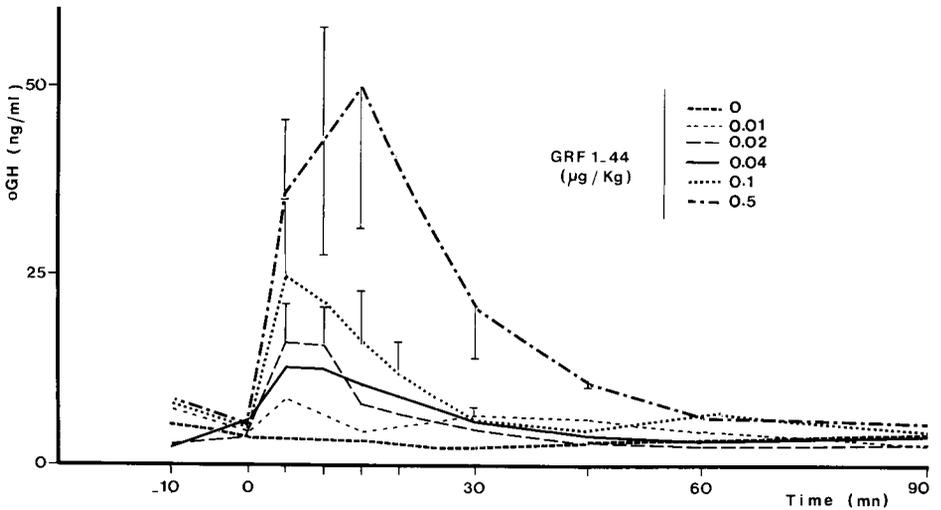


FIG. 3. — GH dose-response in suckling male lambs (5-6 week old) given single iv bolus injections of GRF 1-44 (0-0.5  $\mu\text{g}/\text{kg}$ ; 0-100 pmole/kg). GRF was injected at zero time. Mean  $\pm$  SEM (n = 5).

the injection (fig. 2b). The amplitude of GH response to GRF injection was greater in lambs having higher spontaneous GH peaks than in those having fewer GH peaks and of lower amplitude (fig. 2b, c vs fig. 2a, d). No differences were observed between males and females, each sex demonstrating wide variations in spontaneous or GRF-induced secretory patterns of GH.

Figure 3 shows the effects of increasing doses of GRF 1-44 on GH release in suckling lambs. Manipulation of the animals and injection of the vehicle alone did not produce any GH release. Few lambs responded to a dose of 0.01  $\mu\text{g}/\text{kg}$  (2 pmoles/kg) of GRF, but all responded to higher doses in a dose-dependent manner. When GRF was given at a dose of 0.5  $\mu\text{g}/\text{kg}$  (100 pmoles/kg), the amplitude of the GH peak was  $50 \pm 19$  ng/ml ( $m \pm \text{SEM}$ ) and the subsequent return to basal levels occurred 45 to 60 min after the injection. We observed that the animals which responded to the dose of 0.01  $\mu\text{g}/\text{kg}$ , responded the best to the higher doses.

The variations with time of GH response to GRF 1-44 and GRF 1-29 (0.75 nmole/kg) injected twice daily for 21 days in newborn lambs is depicted in figure 4. Basal plasma GH levels in controls varied between 2.3 and 12.4 ng/ml but did not change significantly during the course of the experiment. Plasma GH levels reached  $68.2 \pm 19.0$  and  $43.2 \pm 10.5$  ng/ml on the first day of treatment (d1) after injection of GRF 1-29 and GRF 1-44, respectively. These levels increased

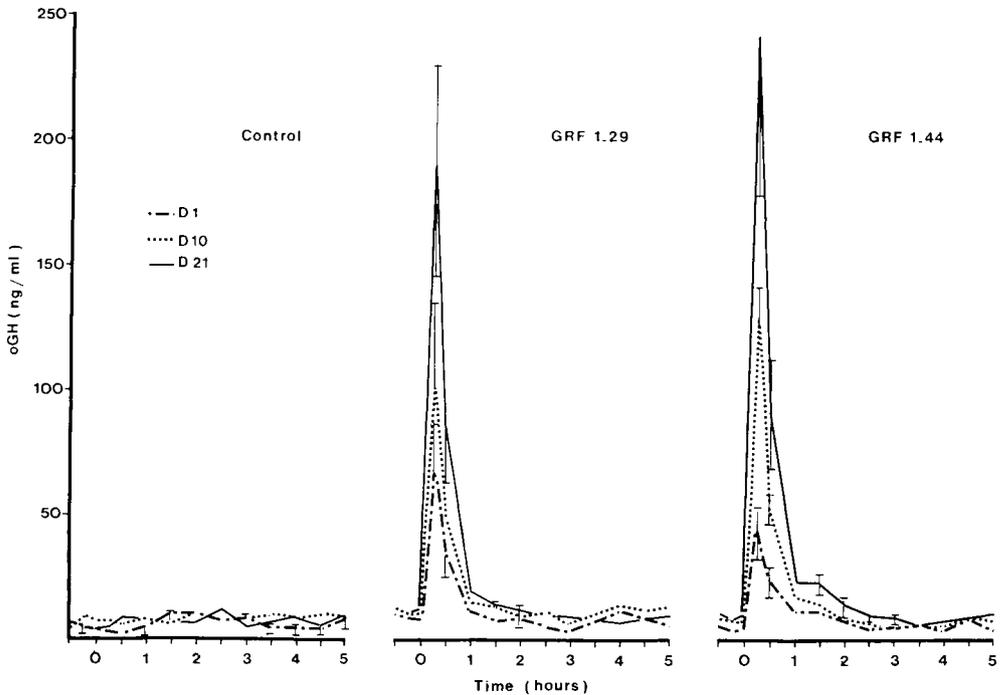


FIG. 4. — GH response to GRF at days 1, 10 and 21 in newborn male lambs that received a sc bolus injection of GRF 1-44 and GRF 1-29 (0.75 nmole/kg) twice daily for 21 days. Every day, GRF was administered at 8 a.m. and 8 p.m. and blood samples were drawn beginning 30 min before the 8 a.m. injection. Controls received the vehicle only. Mean  $\pm$  SEM ( $n = 5$ ).

to  $100.0 \pm 35.4$  and  $127.4 \pm 14.1$  ng/ml on day 10 and to  $188.4 \pm 41.6$  and  $241.5 \pm 63.2$  ng/ml on day 21, following GRF 1-29 and GRF 1-44 injection, respectively. The area under the curve that was plotted for GH concentration against time, increased significantly ( $p < 0.02$ ) from d1 to d21 for both GRF 1-29 and GRF 1-44 groups (table 1). There was no difference between GH response to GRF 1-29 and GRF 1-44 at any stage of treatment.

TABLE 1  
*Effect of a chronic treatment with GRF 1-44 and GRF 1-29 on GH response in lambs.*

Days	Control	GRF 1-44	GRF 1-29
D 1	$5.9 \pm 1.2$	$16.2 \pm 3.0$	$21.3 \pm 5.3$
D 10	$7.6 \pm 0.8$	$34.6 \pm 4.3^*$	$29.9 \pm 7.7$
D 21	$7.7 \pm 0.8$	$61.1 \pm 13.8^{**}$	$48.8 \pm 7.1^{**}$

Data are expressed as ng.h.ml<sup>-1</sup>. They were obtained by measuring the area under the line that was plotted for GH concentration against time after GRF injection. Results, given as mean  $\pm$  SEM ( $n = 5$ ), were statistically analysed by analysis of variance (ANOVA) followed by Duncan's multiple range test.

Significantly different from controls : \*  $p < 0.01$  ; \*\*  $p < 0.02$ .

## Discussion.

The goals of this study were to examine whether lambs taken at two different postnatal stages, when GH secretory patterns were markedly different, would respond to GRF action. In addition, we have shown that GRF 1-44 and GRF 1-29 are equipotent stimulators of GH release. In fact, every GRF analog greater than the 1-27 sequence has been found to be equipotent to GRF 1-44 (Wehrenberg *et al.*, 1983 ; Losa *et al.*, 1984 ; Hart *et al.*, 1985).

The typical response for both of the age group studied is that plasma GH concentrations reach maximum levels during the first minute which follows GRF injection. They remain elevated for approximately 30 min then decrease to basal levels. The pattern of GH secretion is multiphasic : when the animals received a high dose of GRF (2  $\mu$ g/kg), we consistently observed 4-6 episodes of GH secretion ; the number of secretory episodes tended to decrease with lower doses of GRF. Since GRF levels have been found to decrease in the circulation after an equilibration phase of 8 min (Frohman *et al.*, 1984), the amount of exogenous GRF injected may have been enough to restimulate the pituitary gland several times. Because we saw several episodes of GH secretion and not one single prolonged response, this would suggest that the pituitary is not constantly responsive to GRF.

A biphasic pattern of GH secretion following GRF administration has been reported previously in men (Vance *et al.*, 1984) and steers (Moseley *et al.*, 1984), although the two peaks of GH were separated by about 2 hours. Because in this experiment the interval between the different peaks is only a few minutes, it is obvious that we are describing a different phenomenon. In the lamb, endogenous

GH peaks have been described previously as monophasic (Davis *et al.*, 1977). However, our use of more frequent blood sampling periods has allowed us to observe multiphasic episodic secretory peaks. Using the same technique, Laurentie *et al.* (1987) described the same phenomenon during the nycthemeral rhythm of GH secretion in ruminant lambs.

In weaned lambs, the GH response to exogenous GRF (0.1  $\mu\text{g}/\text{kg}$ ) had the same amplitude and duration as spontaneous peaks of GH. The presence of a spontaneous pulse of GH just before or at the time of an injection of GRF did not seem to impair pituitary responsiveness. Likewise, the induction of a GH peak of physiological amplitude with GRF did not prevent the release of a spontaneous pulse within the next 60 min. Thus, it appears that spontaneous and GRF-induced secretory episodes do not interfere with each other and that induction of a GH pulse does not disturb the endogenous rhythm of GH secretion over a period of at least one hour.

The animals with spontaneous GH peaks of higher amplitude responded best to GRF. Inversely, those which had spontaneous peaks of low amplitude responded to GRF with GH peaks of low amplitude. This suggests that some lambs are less responsive to GRF, whether it is exogenous or endogenous. However, we do not know why there was a so much variability between animals since they were apparently under the same environmental and physiological conditions.

As reported in other species, there is a dose-response effect of GRF on GH secretion in sheep. When injected subcutaneously (data not shown), there is also a dose-response effect but in a range of 2-20  $\mu\text{g}/\text{kg}$ . This is consistent with results obtained in men (Sassolas *et al.*, 1985 ; Evans *et al.*, 1985). When injected intravenously, the doses of GRF required to stimulate GH release are 10 to 100 times lower than subcutaneously. We find that the threshold of responsivity to *iv* injections of GRF is between 0.01 and 0.02  $\mu\text{g}/\text{kg}$  in suckling lambs but about 0.04  $\mu\text{g}/\text{kg}$  in weaned lambs (M. Duclos, personal communication). This agrees with a previous report that GH response and sensitivity to GRF action decrease with advancing age in ruminants (Johke *et al.*, 1984). Using the same hGRF 1-44 (SANOFI), Boissel *et al.* (1986) found that the lowest effective *iv* dose was between 0.03 and 0.07  $\mu\text{g}/\text{kg}$  in healthy adult men. Similar results have also been reported in steers (Moseley *et al.*, 1984). In all of the species cited above, including lambs, the maximal effective dose of GRF is 0.5-1  $\mu\text{g}/\text{kg}$ . Similarities among species concerning GRF dose-response effect have been confirmed in pituitary cell cultures (Law *et al.*, 1984 ; Blanchard *et al.*, 1987 ; Brazeau *et al.*, 1982).

Finally, the most important difference between lambs and other species concerns the duration of the GH peak. In lambs, it never lasts longer than 1 hour, whatever the dose or the route of administration, while in men it may last 2 or 3 hours (Sassolas *et al.*, 1985). In addition, we do not observe the rebound described in bovines (Moseley *et al.*, 1984 . McCutcheon *et al.*, 1984).

The significant increase of GH response to GRF during the course of a chronic treatment was unexpected. The reasons for such an increased responsivity of the animals are not understood. Pastoureau *et al.* (1987) have recently reported an increase in plasma somatomedin-C levels in hypotrophic lambs after 45 days of a comparable treatment. Therefore, the increased GH response in lambs chroni-

cally treated with GRF may represent a significant effect of the releasing factor rather than a physiological event. This study demonstrates that suckling lambs are extremely responsive to GRF action. Thus, it is likely that the perinatal decrease in plasma GH levels in sheep is due to very low GRF secretion rather than to a refractory or inhibitory mechanism.

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**Résumé.** *Caractéristiques de la réponse de la GH à l'administration de GRF chez l'agneau.*

Nous avons étudié l'effet de l'administration de GRF 1-44 ou de GRF 1-29 chez des agneaux pris à deux stades physiologiques différents et ayant des profils de sécrétion de la GH différents : des agneaux allaités (âgés de 5-6 semaines) ne présentant pas de décharges épisodiques de la GH (niveaux de base : 5-10 ng/ml) et des agneaux sevrés (âgés de 14-15 semaines) qui sécrètent la GH par épisodes (amplitude des pics : 15-65 ng/ml).

L'injection de GRF par voie *iv* stimule instantanément la sécrétion de la GH. Dans chaque cas, la GH plasmatique atteint son niveau maximum 1-4 min après l'injection de GRF. Le pic sécrétoire est multiphasique et dure 30-60 min. L'administration de GRF (0.1 µg/kg) chez l'agneau sevré induit des pics de GH dont l'amplitude et la durée sont comparables à celles des pics spontanés. L'induction d'un pic de GH par le GRF se produit même lorsque l'injection du GRF est pratiquée après ou pendant un pic spontané de GH. Inversement, on observe des pics spontanés de GH pendant l'heure qui suit l'induction d'un pic de GH par le GRF.

Chez l'agneau allaité, l'injection de GRF par voie intraveineuse dans une gamme allant de 0.01 à 0.5 µg/kg (2 à 100 pmoles/kg) stimule la sécrétion de la GH de façon dépendante de la dose injectée.

La réponse à l'administration biquotidienne de 750 pmoles/kg de GRF 1-44 ou de GRF 1-29 pendant 21 jours chez l'agneau nouveau-né augmente significativement ( $p < 0.001$ ) au cours du traitement. En revanche les réponses au GRF 1-44 et au GRF 1-29 sont identiques.

Ces résultats indiquent que l'agneau est extrêmement sensible au GRF que ce soit avant ou après le sevrage et qu'il n'y a pas de désensibilisation apparente de l'hypophyse après une administration aiguë ou chronique de GRF.

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