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PLASMA LUTEINIZING HORMONE RESPONSE TO INCREASING DOSES OF SYNTHETIC GONADOTROPHIN-RELEASING HORMONE IN HEIFERS

By

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MIKÉL JENSEN, A., K. MYRUP PEDERSEN, J. F. AGGER and A. MADEJ: *Plasma luteinizing hormone response to increasing doses of synthetic gonadotrophin-releasing hormone in heifers.* Acta vet. scand. 1983, 24, 211—224. — The dose-response relationship for a synthetic gonadotrophin-releasing hormone (GnRH) was studied in normally cycling heifers using the area under the luteinizing hormone (LH) curve as a response parameter. Oestrus was synchronized by an injection of 0.5 mg cloprostenol before the experiment started and after the 3rd treatment with GnRH. Treatment with GnRH as assigned in a Latin square included 5 dose levels (0, 10, 50, 100, 250 μg) and 5 treatment days over a period of 22 days. GnRH was capable of inducing an increase of plasma LH within 30 min after injection. Plasma LH response increased with increasing doses of GnRH, the largest increase being observed when the dose was raised from 50 μg to 100 μg. One heifer did not respond to any of the doses applied. The existence of an individual treshold dose of GnRH is suggested.

GnRH; LH; heifers; dose-response curve; Latin square.

The synthetic decapeptide, known as gonadotrophin-releasing hormone (GnRH), is identical with the natural hypothalamic releasing hormone in structure and acts on the anterior pituitary to initiate a release of pituitary gonadotrophins. GnRH has been used extensively with varying results in cattle to induce luteinization of ovarian follicles and ovulation from growing follicles. *Mauer & Rippel* (1972) found that GnRH administration after

progesterone withdrawal resulted in ovulation in heifers, and the first report to demonstrate GnRH-induced release of LH in heifers was published by *Zolman et al.* (1973). Others have reported that GnRH treatment would induce ovulation in milking cows during the early postpartum period (*Britt et al.* 1974, *Zaied et al.* 1980, *Foster et al.* 1980) and in suckling cows (*Troxel et al.* 1980, *Kesler et al.* 1980). GnRH has been used successfully for initiating oestrus in cows with ovarian follicular cysts (*Kittok et al.* 1973, *Cantley et al.* 1975, *Bierschwal et al.* 1975, *Kesler et al.* 1978, *Bäckström et al.* 1980, *Pedersen* 1982). In most experiments the doses of GnRH have been of a wide range owing to uncertainty about the proper dosage. The effect of the treatment has been evaluated through clinical examinations and often been related to the plasma LH response, which has usually been estimated as the peak value and the duration of the LH surge. Only few authors (e.g. *Kaltenbach et al.* 1974) have calculated the area under the LH curve, although that area might be a better biological measure of the response.

The objective of the experiment reported on in the present paper was to study the GnRH dose-response relationship for doses up to 250 μg using the area under the LH curve as a response parameter.

MATERIALS AND METHODS

Experimental design

Five heifers (3 Red Danish and 2 Danish Jersey) were selected for the experiment on the criterion of having a palpable corpus luteum. The luteal phase was confirmed by measuring the plasma progesterone concentration. Twenty-four hours before the beginning of the experiment the heifers were injected with 0.5 mg cloprostenol* intramuscularly (i.m.) to synchronize their oestrous cycles. Treatment with GnRH** as assigned in a Latin square design (Table 1) included 5 dose levels of GnRH and 5 treatment days over a period of 22 days. At the first treatment on Day 1 the heifers were in prooestrus. The second and the third treatment took place on Day 8 (early luteal phase) and Day 12 (mid luteal phase), respectively. Immediately after the third treatment all the heifers were injected with 0.5 mg cloprostenol,

* Estrumate®.

** Nialutin® (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2).

and the fourth and the fifth treatment were carried out on Day 6 (early luteal phase) and Day 10 (mid luteal phase), respectively, following cloprostenol treatment. GnRH was dissolved in physiological saline and injected i.m. The dose levels were 0 (saline control), 10, 50, 100 and 250 μg GnRH. Blood for LH and progesterone analysis was collected in heparinized tubes through a catheter in the jugular vein. Plasma was separated by centrifugation immediately after collection and stored in the frozen state until analysed. Samples were taken immediately before injection of GnRH at time 0, and then at 30 min intervals through the following 8 h. Every other day between treatments, jugular blood was collected to estimate the plasma progesterone level.

Hormone assays

Plasma LH was quantified by double antibody radioimmunoassay with sheep anti-rabbit Ig covalently linked to cellulose as the second antibody, as previously described by *Jensen et al.* (1982). All plasma samples were run in 2 assays. The intra- and interassay coefficients of variation were 2.6 % and 6.5 %, respectively, and the sensitivity was 1.2 ng per ml. Plasma progesterone was measured by a radioimmunoassay described by *Pedersen* (1976) and by *Solti et al.* (1978).

Statistical methods

Analyses comprised 1) simple statistics, 2) response estimation and 3) Latin square analysis (LSA). Simple statistics included the arithmetic mean and the standard error of the mean (s.e.m.) as presented in Figs. 1, 2 and 3.

In the present experiment we measured the total LH response (R) as the area under the LH curve (Fig. 1). Alternative methods to estimate the response area are: 1: The total area under the curve (A_1); 2: the $\log_{10}$ (A_1); 3: the difference between A_1 and A_0; 4: the relative increase (A_1/A_0), and 5: $\log_{10}$ (A_1/A_0). A_1 was the total area under the curve from 0 to 330 min after treatment, and A_0 the area under the LH base level during the same period (Fig. 1):

$$A_1 = \sum_{i=0}^{n} \frac{(LH_i + LH_{i+30})}{2} \times 30 \text{ min}; \quad i = 0, 30, 60, \ldots, 330;$$

$$A_0 = LH_{base} \times 330 \text{ min};$$

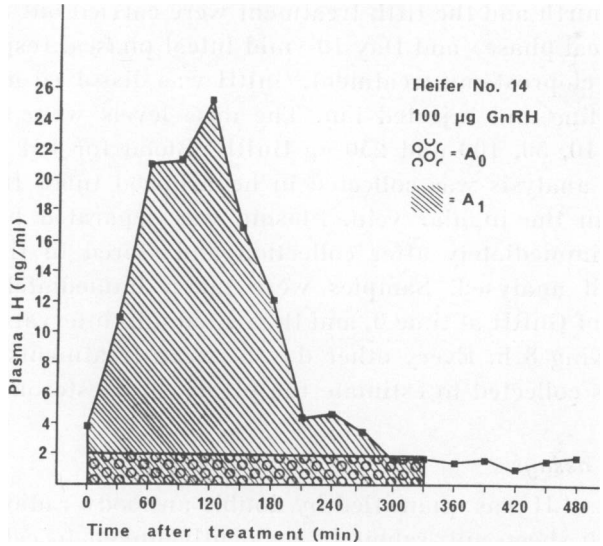

Figure 1. Heifer No. 14, 2. treatment day. Plasma LH concentrations in relation to time after injection of 100 µg GnRH. The areas A_0 and A_1 were used for calculation of the response ($R = \log_{10} (A_1/A_0)$).

The LH base level was defined as the arithmetic mean of the LH values recorded immediately before treatment and those recorded after more than 330 min. After injection of GnRH LH increased and reached pretreatment base levels again within 330 min in most of the 25 treatments. Therefore the standard period from 0 to 330 min was chosen as the response period.

The following results are based on the LH response area calculated as $R = \log_{10} (A_1/A_0)$ as this proved to be the most biologically and statistically suitable method.

The dose-response relationship was evaluated by a Latin square analysis (LSA) (*Snedecor & Cochran* 1967). The method made it possible to control two sources of variation (heifer and date) while we were interested in evaluating the variation due to experimental activity. The design implied that the same subjects (heifers) could be used several times given different levels of treatment.

The model was:

$$R_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk};\ i, j, k = 1, 2, 3, 4, 5;$$

α, β, γ indicating heifer, date (sequence of treatments) and dose (level), respectively.

Table 1. Latin square design for GnRH dose (μg GnRH = D) and LH response (R) by heifer and date of treatment.

Heifer No.	Dose (D) Response (R)	Date of treatment					Sum (R)	$\bar{x}_{i..}$
		7 July	14 July	18 July	24 July	28 July		
10	D	0	250	50	100	10		
	R	0.0639	0.8223	0.5062	0.5760	0.1881	2.1565	0.4313
11	D	10	0	100	250	50		
	R	—0.0517	0.2442	0.2661	0.1746	0.1799	0.8131	0.1626
12	D	50	10	250	0	100		
	R	0.1699	0.2098	0.5644	0.0311	0.2475	1.2227	0.2445
13	D	100	50	0	10	250		
	R	0.6955	0.2272	0.0770	0.1055	0.6385	1.7437	0.3487
14	D	250	100	10	50	0		
	R	0.8673	0.7550	0.0102	—0.0178	0.0394	1.6541	0.3308
Sum (R)		1.7449	2.2585	1.4239	0.8694	1.2934	7.5901	
$\bar{x}_{.j.}$		0.3490	0.4517	0.2848	0.1739	0.2587		

Summary by dose of GnRH.

Dose	0	10	50	100	250	Sum	$\bar{x}...$
Sum (R)	0.4556	0.4619	1.0654	2.5401	3.0671	7.5901	
$\bar{x}_{..k}$	0.0911	0.0924	0.2131	0.5080	0.6134		0.3036

$\bar{x}_{i..}$ = mean response by heifer
$\bar{x}_{.j.}$ = mean response by date
$\bar{x}_{..k}$ = mean response by dose
$\bar{x}...$ = overall mean

A possible effect of the pretreatment plasma progesterone level on the LH response was accounted for in an extended general linear model including the variables heifer, date, GnRH dose and progesterone level (*Helwig & Council* 1979).

RESULTS

An LH response curve was obtained for each of the 25 treatments. An example of the LH response recorded within the period 0 to 480 min after injection of 100 μg GnRH (Heifer No. 14) is shown in Fig. 1. The curve has a characteristic rise and fall within the first 330 min after treatment, with the peak value at 120 min. Fig. 2 demonstrates the mean LH response of the

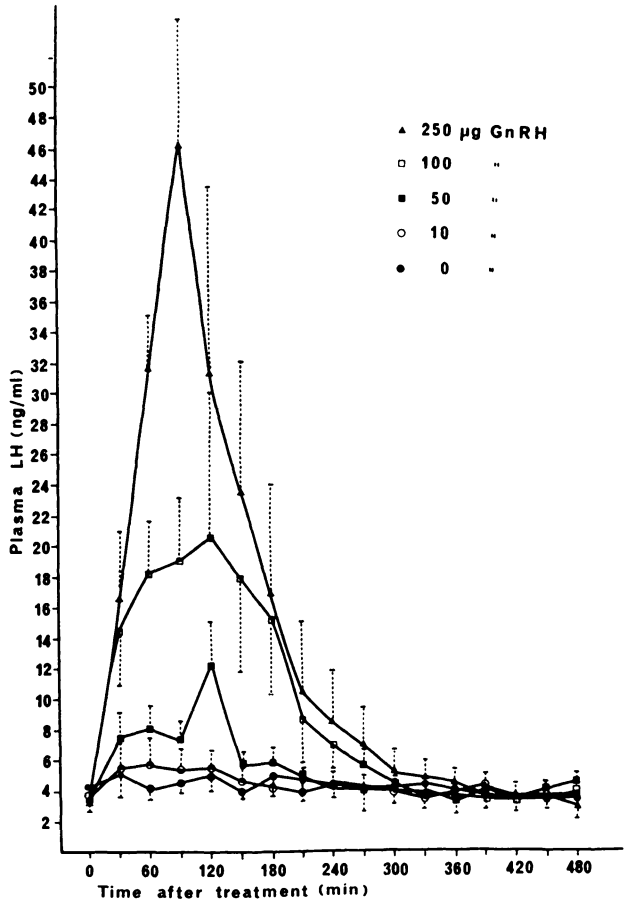

Figure 2. Mean plasma LH concentrations in 5 heifers in relation to time after treatment with, respectively, 0, 10, 50, 100 and 250 µg GnRH i.m. Vertical bars indicate s.e.m.

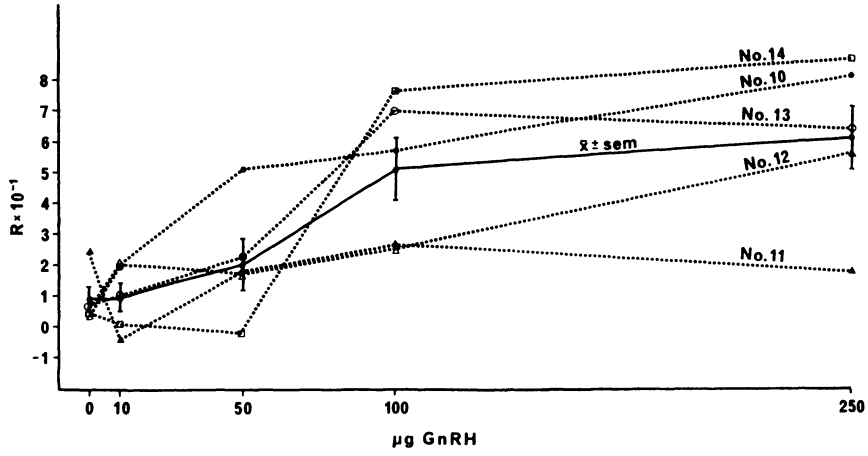

Figure 3. LH response ($R = \log_{10} (A_1/A_0)$) in relation to dosage (0—250 µg GnRH). Dotted lines represent each of the 5 heifers and the solid line represents the mean ($\bar{x} \pm$ s.e.m.).

Table 2. Component analysis in Latin square.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Heifer	4	0.2122	0.0531	1.9516
Date	4	0.2159	0.0540	1.9853
Dose	4	1.1786	0.2947	10.8346***
Error	12	0.3265	0.0272	
Total	24	1.9332		

***: $P < 0.001$.

5 heifers by dose and time after treatment. The mean LH concentration prior to GnRH treatment ranged from 3.3 to 4.4 ng per ml. An increase of LH was observed in the first blood samples taken 30 min after injection of GnRH. Maximum LH values were recorded at 120, 120 and 90 min following injection of 50, 100 and 250 μg GnRH, respectively (Fig. 2). Then LH decreased and reached pretreatment base levels within 330 min. The total LH response values (R) were calculated for all treatments and are presented in Table 1. The data in Table 1 were used for the Latin square analysis. The results (Table 2) indicate no effect of heifer and time sequence of treatment, but a significant effect of the GnRH dose ($P < 0.001$).

Individual and mean dose-response (R) curves for the heifers are presented in Fig. 3. The greatest difference in response was found between 50 and 100 μg GnRH. Apart from the significant effect of the GnRH dose on the LH response area, the s.e.m. values indicate a wide variation among the 5 heifers.

Apparently Heifer No. 11 did not respond to GnRH. After injection of 0, 10, 50, 100 and 250 μg GnRH in this heifer the maximum LH concentrations were 5.4, 5.3, 8.0, 6.0 and 8.7 ng per ml, respectively.

For each of the 5 treatment days plasma progesterone concentrations before GnRH injection and 8 h after are given in Table 3. Oestrus was observed 48 h after synchronization in Heifers Nos. 10, 11, and 12 and after 72 h in Heifers Nos. 13 and 14. So the heifers were in prooestrus on the first treatment day and had low plasma progesterone concentrations. Two days before the second treatment, progesterone had started to increase, and at the third treatment a further elevation was noticed. Fol-

Table 3. Plasma progesterone concentrations in 5 heifers before and 8 h after treatment with GnRH, and the phase of oestrous cycle in which the GnRH treatments took place. The heifers were treated with 0.5 mg cloprostenol on Day 0 and after blood collection on Day 12.

Treatment No.	Day after cloprostenol	Plasma progesterone nmol/l (mean ± s.e.m.) before	8 h after	Phase of oestrous cycle
1	1	1.0 ± 0.2	0.8 ± 0.1 (n.s.)	prooestrus
2	8	2.3 ± 1.2	2.9 ± 1.3 (n.s.)	early luteal phase
3	12	4.8 ± 1.3	8.9 ± 2.1 ($P<0.05$)	mid luteal phase
4	6	3.0 ± 1.3	2.4 ± 1.2 (n.s.)	early luteal phase
5	10	3.2 ± 1.5	4.0 ± 1.9 (n.s.)	mid luteal phase

n.s.: Differences not statistically significant.

lowing the second cloprostenol injection oestrus was observed 48 h later in Heifers Nos. 10 and 11 and 10 days later in Heifer No. 12. Heifers Nos. 13 and 14 did not exhibit oestrus. Heifers Nos. 10 and 11 had very low progesterone levels on the fourth treatment day, i.e., 6 days after the second synchronization, but had reached higher levels by the last treatment day. In Heifers Nos. 12 and 14 a steep increase was recorded at the fourth treatment, while a decrease was noted at the last treatment i.e., 10 days after the second synchronization. In Heifer No. 13 the progesterone level remained elevated in spite of cloprostenol treatment. In heifers in the mid luteal phase an increase in plasma progesterone was observed 8 h after GnRH injections, whereas a slight decrease was noticed if the injection was given during prooestrus or in the early luteal phase (treatment no. 1 and 4). The general linear model procedure, accounting for the variables heifer, date of treatment, GnRH dose, and progesterone level prior to each GnRH treatment, revealed no statistically significant effect of the progesterone level on the LH response (R).

DISCUSSION

The heifers in this experiment were mostly treated during dioestrus in order, if possible, to achieve data that would indicate the minimum GnRH dose capable of inducing a plasma LH surge in the presence of functional luteal tissue. Such a condition was

found quite often when the indication for treatment was a clinical diagnosis of cystic ovarian disease (*Pedersen* 1982). The endogenous milieu of steroid hormones affects the pituitary release of LH (*Convey* 1973), as was demonstrated by *Kaltenbach et al.* (1974) and by *Schams et al.* (1974) who reported that the low response to GnRH during the luteal phase of the oestrous cycle might be due to high endogenous levels of progesterone exerting an inhibitory influence on LH.

The base level of LH recorded in this experiment was higher than reported elsewhere (*Schams & Karg* 1969, *Kaltenbach et al.* 1974) owing to an assay technique involving a shorter incubation time. Injection of GnRH (except for the 10-μg dose) resulted in LH surges that resembled the LH surges reported by *Kaltenbach et al.* (1974) and by *Sequin et al.* (1976) in terms of magnitude, duration and shape. However, the duration of the GnRH-induced LH peaks were 1—2 h shorter than the preovulatory LH surge (*Schams & Karg* 1969, *Dobson* 1978, *Rahe et al.* 1980).

Plasma LH response has hitherto usually been evaluated as the peak value and the duration of the LH surge. In the present experiment we measured the LH response as the relative increase of the area under the LH curve representing the period from 0 to 330 min after treatment (Fig. 1). Alternative methods for response calculation were mentioned under materials and methods. To be biologically correct we had to consider the response area in relation to the pretreatment LH levels so results from using A_1 and $\log_{10}(A_1)$ are not further presented. The same result was obtained from LSA using $(A_1 - A_0)$, (A_1/A_0) and $\log_{10}(A_1/A_0)$; i.e. an effect of dose only. However, the log transformation $R = \log_{10}(A_1/A_0)$ was used as it gave an approximately constant residual variance. Furthermore it gave the relative increase in the area as it corresponded to changes relative to an individually varying LH base level.

There was no effect of the progesterone pretreatment level in the extended general linear model procedure regardless of whether the response area was expressed as $(A_1 - A_0)$ or as $\log_{10}(A_1/A_0)$.

In Heifer No. 11 no response to GnRH treatment was observed, possibly because of an LH elevation of very short duration as was also reported by *Kalra et al.* (1974) who collected blood at 10-min intervals and observed a plasma LH increase of shorter duration than 30 min in luteal-phase heifers. The sampling inter-

val in the present study was 30 min, and the short duration of the LH surge emphasizes the importance of frequent sampling.

Kaltenbach et al. (1974) found little or no increase in serum levels of LH when an endogenous LH release had preceded the GnRH injection. But a depletion of pituitary LH could not explain the missing LH response in Heifer No. 11 during dioestrus. *Madej et al.* (1980) found that heifers with ovarian disorders had an almost significantly lower response than normal heifers. However, before the treatment with GnRH Heifer No. 11 had shown plasma progesterone concentrations that were consistent with the assumed time of oestrous cycle, indicating a functional corpus luteum.

The present experiment was carried out also to support a clinical trial on the use of different dose levels of GnRH for treatment of cystic ovarian disease in dairy cows. The trial revealed no statistically significant differences in clinical response between animals injected with 50, 100 and 250 μg GnRH, respectively (*Pedersen* 1982). The possible existence of some kind of treshold dose would be of interest with a view to working out formula for clinical use of GnRH preparations, and the experimental results presented here would seem to suggest that a treshold dose for bringing about an LH release may in fact be found between 50 and 100 μg GnRH. The data given in Fig. 3 might be taken to reflect the existence of an individual treshold dose. For Heifer No. 10 the treshold dose would be between 10 and 50 μg GnRH, for Heifers Nos. 13 and 14 between 50 and 100 μg, for Heifer No. 12 between 100 and 250 μg, and for Heifer No. 11 over 250 μg. The observed response pattern may be interpreted to show that by increasing dose of GnRH a greater proportion of animals responds to treatment rather than an increased response in the individual animals. This hypothetic pattern of dose-response relationship does not necessarily contradict the notion that some degree of linearity may exist between dose and response both before and after reaching the treshold dose; in fact this would be consistent with biological principles.

A positive relationship between LH and progesterone release was found in the dioestrous heifers, whereas no progesterone response was demonstrated during prooestrus. This is in agreement with findings by *Hoffmann et al.* (1974) who demonstrated that LH is the main luteotrophic factor in the cow when luteal tissue is present in the ovaries.

CONCLUSIONS

a) By intramuscular injection of GnRH it was possible to induce an increase of plasma LH within 30 min, and the elevated LH level would persist for 4—5½ h.

b) The plasma LH response increased with increasing doses of GnRH.

c) The greatest increase was found when the dose was raised from 50 to 100 μg GnRH, indicating that a dose of 100 μg would be preferable to one of 50 μg.

d) Some heifers (in this experiment 1 out of 5) may not respond at all to the injection of GnRH in doses of 250 μg or less.

e) There is evidence to suggest the existence of individual treshold doses which leads to the hypothesis that increasing the dose of GnRH may result in a greater proportion of animals responding rather than in an increased response in the individual animals.

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REFERENCES

Bierschwal, C. J., H. A. Garverick, C. E. Martin, R. S. Youngquist, T. C. Cantley & M. D. Brown: Clinical response of dairy cows with ovarian cysts to GnRH. J. Anim. Sci. 1975, *41*, 1660—1665.

Britt, J. H., R. J. Kittok & S. D. Harrison: Ovulation, estrus and endocrine response after GnRH in early postpartum cows. J. Anim. Sci. 1974, *39*, 915—919.

Bäckström, G., L.-E. Edqvist & A. Lillo: GnRH and HCG for treatment of cystic ovaries in the cow: Evaluation of a comparative field study. 9th. Int. Congr. Anim. Repr. and A.I., Madrid 1980, *4*, 647—649.

Cantley, T. C., H. A. Garverick, C. J. Bierschwal, C. E. Martin & R. S. Youngquist: Hormonal responses of dairy cows with ovarian cysts to GnRH. J. Anim. Sci. 1975, *41*, 1666—1673.

Convey, E. M.: Neuroendocrine relationships in farm animals: A review. J. Anim. Sci. 1973, *37*, 745—757.

Dobson, H.: Plasma gonadotrophins and oestradiol during oestrus in the cow. J. Reprod. Fert. 1978, *52*, 51—53.

Foster, J. P., G. E. Lamming & A. R. Peters: Short-term relationships between plasma LH, FSH and progesterone concentrations in post-partum dairy cows and the effect of Gn-RH injection. J. Reprod. Fert. 1980, *59*, 321—327.

Helwig, J. T. & K. A. Council: SAS User's Guide. SAS Institute Inc., Raleigh, North Carolina, USA, 1979, 494 pp.

Hoffmann, B., D. Schams, R. Bopp, M. L. Ender, T. Giménez & H. Karg: Luteotrophic factors in the cow. Evidence for LH rather than prolactin. J. Reprod. Fert. 1974, *40*, 77—85.

Jensen, A. Mikél, T. Greve, A. Madej & L.-E. Edqvist: Endocrine profiles and embryo quality in the PMSG-$PGF_{2\alpha}$ treated cow. Theriogenology 1982, *18*, 33—44.

Kalra, P. S. & W. W. Thatcher: Effect of GnRH in $PGF_{2\alpha}$ pretreated heifers. J. Anim. Sci. 1974, *39*, 214.

Kaltenbach, C. C., T. G. Dunn, T. E. Kiser, L. R. Corah, A. M. Akbar & G. D. Niswender: Release of FSH and LH in beef heifers by synthetic gonadotrophin releasing hormone. J. Anim. Sci. 1974, *38*, 357—362.

Kesler, D. J., H. A. Garverick, A. B. Caudle, C. J. Bierschwal, R. G. Elmore & R. S. Youngquist: Clinical and endocrine responses of dairy cows with ovarian cysts to GnRH and $PGF_{2\alpha}$. J. Anim. Sci. 1978, *46*, 719—725.

Kesler, D. J., T. R. Troxel & D. L. Hixon: Effect of days postpartum and exogenous GnRH on reproductive hormone and ovarian changes in postpartum suckled beef cows. Theriogenology 1980, *13*, 287—296.

Kittok, R. J., J. H. Britt & E. M. Convey: Endocrine response after GnRH in luteal phase cows and cows with ovarian follicular cysts. J. Anim. Sci. 1973, *37*, 985—989.

Madey, A., B. Barcikowski, R. Stupnicki, E. Kula & Z. Binienda: Luteinizing hormone response to injection of synthetic gonadotrophin releasing hormone or infusion of oestradiol-17 beta in heifers. Anim. Reprod. Sci. 1980, *3*, 17—21.

Mauer, R. E. & R. H. Rippel: Response of cattle to synthetic gonadotropin releasing hormone. J. Anim. Sci. 1972, *35*, 249.

Pedersen, H.: Plasmaprogesteron ved brunstsynkronisering og superovulation af kvier målt ved radioimmunoassay. (Plasma progesterone after synmhronization of heifers measured by radioimmunoassay). Kgl. Vet.- og Landbohøjsk., Inst. Sterilitetsforskn. Årsberetn. 1976, *19*, B13—B24.

Pedersen, K. M.: Treatment of cystic ovarian disease in the dairy cow with 50, 100 or 250 μg of gonadotropin-releasing hormone (GnRH, LHRH). Nord. Vet.-Med. 1983, *35*, 18—27.

Rahe, C. H., R. E. Owens, J. L. Fleeger, H. J. Newton & P. G. Harms: Pattern of plasma luteinizing hormone in the cystic cow: Dependence upon the period of the cycle. Endocrinology 1980, *107*, 498—503.

Schams, D. & H. Karg: Radioimmunologische LH-Bestimmung im Blutserum vom Rind unter besonderer Berücksichtigung des Brunstzyklus. (Radioimmunoassay of LH in bovine blood serum with special regard to the oestrous cycle). Acta endocr. 1969, *61*, 96—103.

Schams, D., F. Höfer, E. Schallenberger, M. Hartl & H. Karg: Pattern of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in bovine blood plasma after injection of a synthetic gonadotropin-releasing hormone (Gn-RH). Theriogenology 1974, *1*, 137—151.

Sequin, B. E., E. M. Convey & W. D. Oxender: Effect of gonadotropin-releasing hormone and human chorionic gonadotropin on cows with ovarian follicular cysts. Amer. J. vet. Res. 1976, *37*, 153—157.

Snedecor, G. W. & W. G. Cochran: Statistical Methods. Iowa State Univ. Press, Ames, Iowa, USA 1967, 593 pp.

Solti, L., T. Greve & H. H. Koefoed-Johnsen: Plasma progesterone assays in superovulated cattle. Acta vet. scand. 1978, *19*, 298—309.

Troxel, T. R., D. J. Kesler, R. C. Noble & S. E. Carlin: Ovulation and reproductive hormones following steroid pretreatment, calf removal and GnRH in postpartum suckled beef cows. J. Anim. Sci. 1980, *51*, 652—659.

Zaied, A. A., H. A. Garverick, C. J. Bierschwal, R. G. Elmore, R. S. Youngquist & A. J. Sharp: Effect of ovarian activity and endogenous reproductive hormones on GnRH-induced ovarian cycles in postpartum dairy cows. J. Anim. Sci. 1980, *50*, 508—513.

Zolman, J., E. M. Convey, J. H. Britt & H. D. Hafs: Release of bovine luteinizing hormone by purified porcine and synthetic gonadotropin releasing hormone. Proc. Soc. exp. Biol. Med. 1973, *142*, 189—193.

SAMMENDRAG

Plasma luteiniserende hormon respons hos kvier efter stigende doser af syntetisk gonadotropin-releasing hormon.

Det syntetiske gonadotropin-releasing hormon (GnRH, Nialutin®) anvendt i dette forsøg er af identisk struktur med det naturlige releasinghormon i hypothalamus og påvirker hypofyseforlappen til udskillelse af gonadotropiner. GnRH har været anvendt til at inducere luteinisering af follikler, til igangsætning af østralcyklus hos køer med

follikelcyster og til at fremkalde ovulation hos køer i den tidlige postpartum periode. Dosisstørrelse har været meget varierende, og behandlingseffekten har oftest været vurderet på basis af en klinisk undersøgelse sammenholdt med gonadotropin-udskillelsen, bl. a. målt som den maksimale udskillelse af luteiniserende hormon (LH toppen) og varigheden af denne top. Et andet og måske mere korrekt biologisk mål for LH respons er arealet under LH kurven, og formålet med dette forsøg var at studere GnRH dosisrespons for stigende doser GnRH ved at benytte en arealberegning som responsparameter.

GnRH behandlingerne blev foretaget såvel i proøstrus som i diøstrus, da tidligere forsøg har vist at follikelcyster ofte indeholder luteinvæv. I forsøget indgik 5 kvier med normal østralcyklus. Cyklus blev synkroniseret med 0,5 mg cloprostenol (Estrumate®). Behandling med 0, 10, 50, 100 og 250 μg GnRH blev foretaget på 5 forsøgsdage fordelt over en periode på 22 dage. GnRH injektion medførte en stigning i plasma-LH i løbet af 30 min og LH niveauet forblev højt i 4—5½ time. Plasma-LH responset, målt som arealet under LH kurven, steg med stigende dosis GnRH. Den største stigning fandtes, når dosis steg fra 50 til 100 μg GnRH.

En af forsøgskvierne svarede ikke på GnRH behandlingerne. Mulighed for forekomst af en individuel GnRH tærskeldosis er omtalt og fører til den hypotese, at stigende GnRH dosis snarere medfører, at en større andel af dyrene svarer på en behandling, end at det enkelte dyr giver større respons.

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