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OVULATION AND ENDOCRINE RESPONSE AFTER LH-RH IN DOMESTIC ANIMALS (1)

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SUMMARY

Endocrine and ovulatory responses were examined after luteinizing hormone-releasing hormone (LH-RH) administration in cows with ovarian follicular cysts, in diestrous cows, in cows 2 weeks after parturition and in diestrous and proestrous heifers. Serum LH concentrations increased after LH-RH treatment and the greatest response was observed in cows with ovarian follicular cysts. Serum progesterone concentrations were increased at 6 hours after LH-RH in animals which had functional luteal tissue in the ovaries but serum estradiol and estrone were unchanged. Ovarian follicular cysts luteinized after LH-RH treatment in cows and estrous cycles were initiated in these animals. Ovulation was induced 1 day after LH-RH administration in cows 2 weeks after parturition, but was not induced after LH-RH treatment in diestrous heifers and cows or proestrous heifers. Results obtained in the bovine are discussed in relation to results after LH-RH treatment in ewes, gilts and mares.

INTRODUCTION

Isolation of porcine (SCHALLY *et al.*, 1971) and ovine (AMOSS *et al.*, 1971) luteinizing hormone/follicle stimulating hormone releasing hormone (LH-RH/FSH-RH) resulted in its rapid structural identification and synthesis (MATSUO *et al.*, 1971 *a*, *b*). Purified natural and synthetic LH-RH products were shown to have comparable LH-releasing activity in laboratory animals (SCHALLY *et al.*, 1972 ; BURGUS *et al.*, 1972), sheep (REEVES *et al.*, 1970 ; ARIMURA *et al.*, 1972) and cattle (ZOLMAN *et al.*, 1973). Much of this early work was recently reviewed by CONVEY (1973).

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After these initial studies, we began experiments to determine endocrine and ovulatory responses to LH-RH in cattle. In our studies gonadotropin releasing hormone (GnRH ; Abbott Laboratories, North Chicago, Illinois) was used but will be referred to as LH-RH for uniformity in comparing our results with results of others who may have used different LH-RH preparations.

MATERIALS AND METHODS

Experiment I

Serum hormone and ovarian structural changes after administration of LH-RH to five lactating Holstein cows with ovarian follicular cysts were compared with responses observed in four cows given LH-RH during diestrus (KITTOK *et al.*, 1973). Blood samples were collected on a schedule designed to detect acute and chronic changes in serum hormones after each animal was given three intravenous injections of LH-RH (100 μg) at 2 hours intervals. Serum LH, progesterone and estrogens were quantified by radioimmunoassay and ovarian structural changes were monitored twice weekly by rectal palpation.

Experiment II

Endocrine and ovulatory responses were examined after LH-RH treatment in early post-partum dairy cows (BRITT *et al.*, 1974). Twenty lactating Holstein cows were given LH-RH (100 μg) or saline via a No 5 gelatin capsule implanted in an ear on day 14 post-partum. Blood samples were collected from a jugular vein at frequent intervals from 2 hours before until 6 hours after treatment on day 14 post-partum then on days 16, 18, 21, 29, 31, 32, 33, 34, and 35 post-partum and twice weekly thereafter until day 65 when the experiment terminated. Serum LH, progesterone and estrogens were quantified by radioimmunoassay and changes in ovarian structures were monitored by rectal palpation twice weekly until day 65 post-partum.

Experiment III

The effect of LH-RH on ovulation and estrous cycle length in heifers was examined. Twenty Holstein heifers approximately 14 months of age received no treatment or intramuscular administration of 200 μg LH-RH on day 15, 17 or 19 of an estrous cycle. Blood samples were collected via tail venipuncture on alternate days from day 15 until estrus or development of a new corpus luteum. Ovulation and corpus luteum development were monitored by palpation per rectum. Serum progesterone was quantified by radioimmunoassay according to LOUIS *et al.*, (1973).

RESULTS

Experiment I

Serum LH increased after each dose of LH-RH in luteal phase cows and cows with ovarian follicular cysts (fig. 1). Peak LH response after each dose was two- to four-fold greater in cows with ovarian follicular cysts compared with cows treated during diestrus. Serum progesterone prior to first LH-RH injection averaged 0.9 ± 0.4 ng/ml for cows with ovarian follicular cysts which was less than the comparable average (5.9 ± 0.8 ng/ml) for luteal phase cows. However, mean serum estradiol (9.4 ± 3.1 vs 8.8 ± 0.7 pg/ml) and estrone (3.8 ± 0.5 vs 3.0 ± 0.4

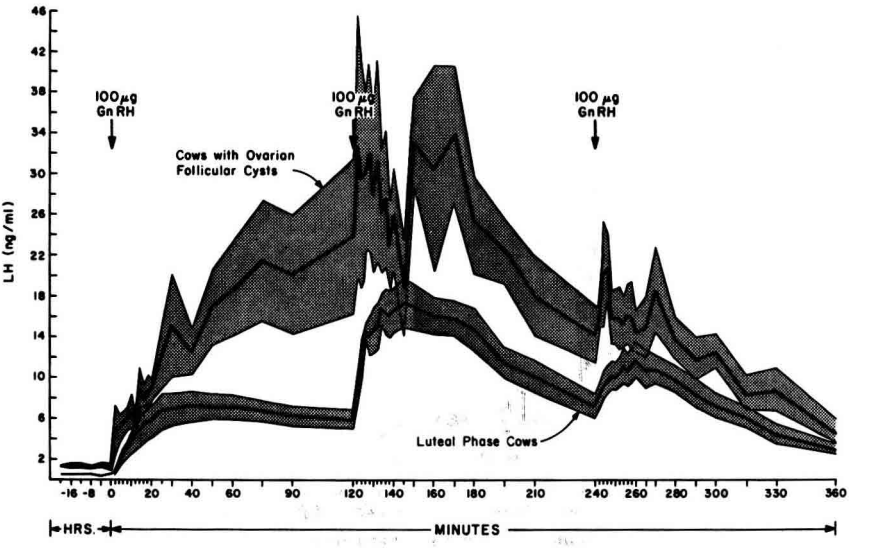

FIG. 1. — *Serum luteinizing hormone response after LH-RH* (GnRH) *in luteal phase cows and cows with ovarian follicular cysts*

(Shaded area represents standard errors of the mean)

pg/ml) were not different in cows with ovarian follicular cysts compared with cows in diestrus. Serum progesterone peaked at 11 days after LH-RH (fig. 2) and then declined according to a pattern characteristic of normal estrous cycles. One of five cows with ovarian follicular cysts ovulated after LH-RH treatment, four developed luteinized follicles and all five cows exhibited estrus 20 to 24 days after treatment. These results suggested that luteal tissue in the follicular cyst assumed the role of a functional corpus luteum in each of four cows in which a palpable corpus luteum was not detected.

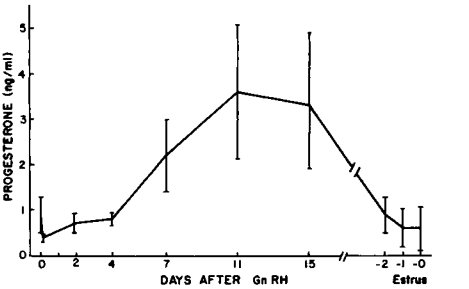

FIG. 2. — *Chronic serum progesterone concentrations after LH-RH* (GnRH) *in cows with ovarian follicular cysts*

(Vertical lines represent standard errors of the mean)

Experiment II

Serum LH in cows given LH-RH on day 14 post-partum increased by 1 hour and remained elevated at 6 hours after treatment (fig. 3). Peak serum LH (15.0 $\pm$ 1.9 ng/ml) occured at approximately 4 hours after LH-RH though blood samples were not collec-

ted frequently enough to detect highest values in all animals. Serum LH concentrations were as high as 24.8 ng/ml in individual samples collected at 4 hours after LH-RH. Serum LH remained unchanged during 6 hours after cows were given gelatin capsule implants containing saline (fig. 3). Serum estrogens and progesterone were not acutely

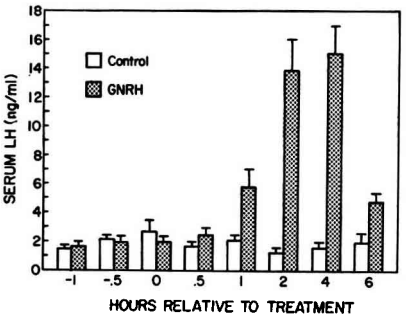

FIG. 3. — *Serum luteinizing hormone response after LH-RH* (GnRH) *or saline to lactating cows on day 14 post-partum*
(Vertical lines represent standard errors of the mean)

affected in cows given LH-RH on day 14 post-partum (table 1). The increase in serum estrone concentrations at 4 hours after LH-RH or saline was due to peaks in individual samples from one or two animals in each group and was not related to LH-RH treatment ($P > .05$).

TABLE I

Serum estrone, estradiol and progesterone at 0 and 4 or 6 hr after 100 µg LH-RH (GnRH) *on day post-partum*

	Estrone [a]		Estradiol [a]		Progesterone [b]	
	0 hr	4 hr	0 hr	4 hr	0 hr	4 hr
Saline	11.0 ± 0.8	19.9 ± 8.3	5.0 ± 0.5	4.3 ± 0.5	0.4 ± 0.1	0.2 ± 0.1
GnRH	11.3 ± 1.0	16.5 ± 8.5	3.8 ± 0.6	3.8 ± 0.6	0.3 ± 0.1	0.3 ± 0.1

a : pg/ml ; $n = 10$.
b : ng/ml ; $n = 9$. One cow which had a functional corpus luteum was excluded.

One cow given LH-RH on day 14 post-partum had ovulated on day 9, all others ovulated on day 15, 1 day after treatment. Thus, the average interval from parturition to first ovulation was 14.4 ± 0.6 days for cows given LH-RH compared with 23.6 ± 2.6 days for eight of 10 cows given saline. Two given saline on day 14 post-partum developed ovarian follicular cysts and did not ovulate prior to day 65 when the experiment terminated. One cow given saline was diagnosed as having luteinized ovarian follicles even though she exhibited estrus at 21 day intervals beginning

20 days after treatment. Progesterone in serum of this cow did not exceed 0.3 ng/ml until day 65 post-partum.

Serum progesterone changes during 3 weeks after LH-RH administration to cows on day 14 post-partum is depicted in figure 4. Although lower in magnitude,

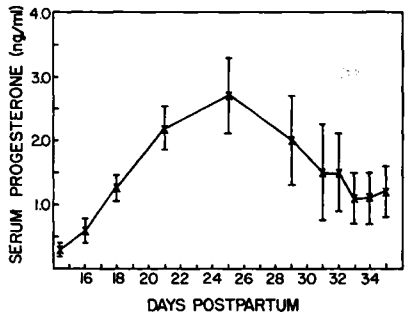

FIG. 4. — *Chronic serum progesterone concentrations after administration of LH-RH* (GnRH) *to lactating dairy cows on day 14 post-partum*

(Vertical lines represent standard errors of the mean.
Data from one cow which had a functional corpus luteum is excluded)

the progesterone profile was similar to that during the bovine estrous cycle. Cows given LH-RH on day 14 post-partum averaged 3.1 ± 0.1 ovulations prior to day 65 post-partum compared with 2.0 ± 0.4 ovulations for cows given saline (fig. 5).

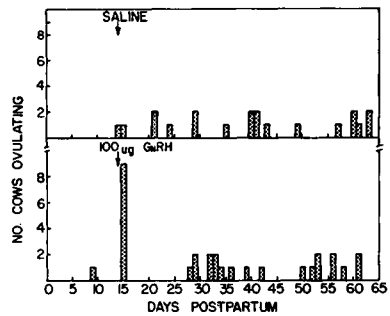

FIG. 5. — *Frequency of ovulation in lactating cows given LH-RH* (GnRH) *or saline on day 14 post-partum*

(*n* = 10 for each treatment group)

The first post-partum ovulation in cows given LH-RH or saline on day 14 post-partum occurred most frequently on the ovary opposite the post-gravid uterine horn and was not affected by treatment. Interval from first to second ovulation in cows given LH-RH was 18.1 ± 1.0 days compared with 20.9 ± 0.9 days for cows given saline. None of the cows given LH-RH exhibited estrus in association with the induced ovulation while 2 of 8 saline-treated cows were detected in estrus just prior to first ovulation. Interval from parturition to first estrus was 41.0 ± 4.0 days for cows given LH-RH on day 14 post-partum compared with 37.5 ± 4.0 days for cows treated with saline. Thus, while LH-RH treatment resulted in a shorter interval from

parturition to first ovulation, it did not shorten the interval to first estrus. However, all cows given LH-RH exhibited regular ovarian cycles until day 65 post-partum while 3 of 10 cows given saline manifested abnormal ovarian activity (ovarian follicular cysts or luteinized follicles) during this period.

Experiment III

Administration of 200 μg LH-RH to heifers on day 15, 17 or 19 of an estrous cycle increased the variation in interval from previous estrus to subsequent estrus and ovulation. All saline injected heifers exhibited estrus prior to ovulation compared with 3 of 5, 4 of 5 and 2 of 5 heifers given LH-RH on day 15, 17 or 19 (fig. 6). The

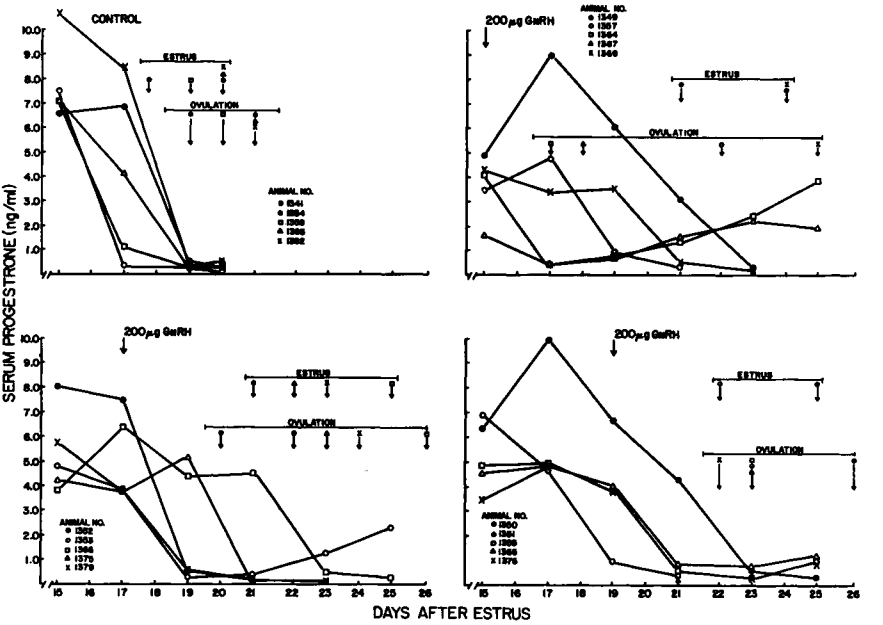

FIG. 6. — *Serum progesterone concentrations, time of estrus and time of ovulation in heifers given LH-RH* (GnRH) *on days 15, 17 or 19 of an estrous cycle*

inter-ovulatory interval in saline injected heifers was 19.4 ± 0.4 days compared with 20.4 ± 1.7, 22.0 ± 1.0 and 22.4 ± 0.7 days for heifers given LH-RH on days 15, 17 or 19. In no case did ovulation occur on the day after LH-RH administration ; however, when LH-RH was given on day 15 of an estrous cycle, day of subsequent ovulation ranged from 17 to 25 of that cycle, compared with 20 to 26 and 22 to 26 for animals given LH-RH on days 17 or 19.

Serum progesterone concentration (ng/ml) averaged 0.3 ± 0.1 on day 19 in saline injected heifers compared with 2.4 ± 1.0, 2.1 ± 1.1 and 3.8 ± 0.9 in heifers given LH-RH on days 15, 17 or 19 (fig. 6). Elevated serum progesterone on day 19 in each of 2 heifers given LH-RH on days 15 or 17 may be attributable to stimulation of luteal progesterone secretion in these animals. Results from heifers treated on day

19 are somewhat biased since only those which had estrous cycles greater than 19 days were included in this group.

DISCUSSION

LH-RH in cattle

Luteinizing hormore release after LH-RH has been examined during various physiological states in the bovine. ZOLMAN *et al.*, (1973) from our laboratory first reported that synthetic and purified porcine LH-RH were capable of inducing LH release from bovine pituitary tissues *in vitro*, and that synthetic LH-RH resulted in a dose-related increase in serum LH concentrations during early diestrus in heifers. Cows with ovarian follicular cysts released more LH after LH-RH treatment than cows treated during diestrus (KITTOK *et al.*, 1973). This indicated that responsiveness of the pituitary to LH-RH varied with physiological state of the animal. Thus ZOLMAN *et al.*, (1974) administered LH-RH to heifers during diestrus or proestrus and observed that serum LH response to LH-RH was positively related to pretreatment serum level of estradiol and estrone ; a greater response occurred in animals with higher estrogen concentrations.

Serum FSH also increased after LH-RH in the bovine and peak response occurred simultaneously with the LH-peak (AKBAR *et al.*, 1974 ; KALTENBACH *et al.*, 1974 ; ZOLMAN and CONVEY, 1973). However, peak serum FSH after LH-RH was only two-to four-fold higher than pretreatment values compared with twenty-fold or more increases in serum LH.

BIERSCHWAL *et al.*, (1974) administered varying doses of LH-RH to 114 cows with ovarian follicular cysts and reported that 74 percent of the treated cows developed luteinized ovarian follicles. A similar response after LH-RH treatment in 15 cows with ovarian follicular cysts was observed by GRUNERT *et al.*, (1973). Thus our initial observation (KITTOK *et al.*, 1973) that LH-RH was effective in initiating estrous cycles in cows with follicular cysts has been verified in clinical studies.

Our observation that LH-RH caused LH release and ovulation in post-partum dairy cows (BRITT *et al.*, 1974) suggests that LH-RH may be used to initiate ovarian cycles in lactating animals. A similar observation was reported by SCHAMS *et al.*, (1973) who gave 1500 μg LH-RH to each of 6 cows between day 12 and 18 post-partum and observed ovulation in all animals 1 day after treatment. However, these authors did not provide data on the time of ovulation during a similar post-partum interval in non-treated cows. Since post-partum anestrus in lactating cows prohibits their response to luteolytic agents such as prostaglandin $F_{2\alpha}$ it is possible that administration of LH-RH can be used to induce LH release and ovulation in order to provide a common physiological state for synchronization of ovulation in these animals.

Apparently ovulation in the bovine can not be induced by treatment with LH-RH if a functional corpus luteum is present. The reason for this lack of response during diestrus is not known but may result from an inhibitory action of progesterone on ovulation.

LH-RH in sheep

REEVES *et al.*, (1970, 1971) first demonstrated that highly purified porcine LH-RH induced LH release in ewes and that a greater response occurred on the day of estrus relative to other days of the estrous cycle. As in the bovine, LH-RH administration to ewes also caused an increase in serum FSH (REEVES *et al.*, 1972; JONAS *et al.*, 1973; SYMONS *et al.*, 1974). Ovulation has been observed after LH-RH treatment in anestrous ewes (REEVES *et al.*, 1972, 1974; HARESIGN *et al.*, 1973; RIPPEL *et al.*, 1974 *b*; KINDER *et al.*, 1974) in lactating ewes (RESTALL and RADFORD, 1974), in diestrous ewes (RIPPEL *et al.*, 1974 *a*, *b*) and in ewes after cessation of progesterone injections (SERGERSON *et al.*, 1974). CRIGHTON *et al.*, (1973) reported that only 3 of 8 ewes had functional corpora lutea 10 to 13 days after induction of ovulation with LH-RH, even though all eight ewes had observable ovulation points two days after treatment. Thus while the LH release after LH-RH resulted in ovulation in anestrous ewes, functional capabilities of the resultant corpora lutea were reduced.

SERGERSON *et al.*, (1974) reported reduced fertility in ewes given LH-RH after synchronization with progesterone. They attributed this reduction in fertility to a block of sperm transport presumably caused by a decrease in estrogen secretion after LH-RH. Thus synchronization of ovulation with LH-RH in combination with other agents may result in reduced fertility if there is an alteration in steroid secretion around estrus.

LH-RH in swine

CHAKRABORTY *et al.*, (1973) observed serum LH peaks in preputertal gilts 15 or 30 min after each of 16 LH-RH injections given at 6 hours intervals. BAKER *et al.*, (1973, 1974) first reported ovulation after LH-RH treatment in prepubertal gilts primed with pregnant mare serum gonadotropin (PMS); however, the percent of gilts ovulating after 1, 5 or 10 mg LH-RH was lower than that observed in PMS-primed gilts given human chorionic gonadotropin (HCG). In contrast, RAMPACEK *et al.*, (1974) found that 15 of 18 PMS-primed prepubertal gilts ovulated when given 125 μg LH-RH at 72 or 91.5 hours after PMS treatment. However, they observed that only 5 of 15 gilts which ovulated and were inseminated were pregnant 25 days later. Based on progesterone concentrations in serum samples collected frequently during two weeks after LH-RH treatment, they concluded that gilts which were non-pregnant failed to maintain functional corpora lutea. Thus the prepubertal gilt like the anestrous ewe may require sustained luteotropic stimulation in order to maintain corpora lutea induced by LH-RH treatment.

LH-RH in the mare

GINTHER and WENTWORTH (1974) gave 400 μg LH-RH to mares in late anestrus or on the second day of estrus and observed a rapid increase in serum LH, but follicular development or interval from the onset of estrus to ovulation was not affected. Similarly, DOWNEY *et al.*, (1974) injected mares with 1 mg LH-RH on the second day of estrus and found no change in the interval from onset of estrus to ovulation

though duration of estrus was reduced by 2 days. However, when they gave mares 2 mg LH-RH on the second day of estrus and on each subsequent day until ovulation the interval from onset of estrus to ovulation and duration of estrus were reduced.

CONCLUSIONS

1. LH-RH is a potent releaser of LH and FSH in domestic animals.

2. Changes in serum hormone concentrations after LH-RH treatment vary with species and physiological state of treated animals.

3. LH-RH can be used to initiate estrous cycles in cows with ovarian follicular cysts.

4. LH-RH treatment causes ovulation in cows when given two weeks after parturition but not when given during diestrus.

Colloque : Control of sexual cycles in domestic animals
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RÉSUMÉ

RÉPONSE ENDOCRINIENNE ET OVULATION
APRÈS ADMINISTRATION DE LH-RH CHEZ LES ANIMAUX DOMESTIQUES

Les réponses endocriniennes et ovulatoires ont été examinées après l'administration de l'hormone activant la sécrétion de LH (LH-RH) à des vaches :

1) ayant des kystes follicullaires ovariens,
2) étant au stade diœstrus,
3) à deux semaines post-partum,
4) à des génisses au stade diœstrus et proœstrus.

Les concentrations en LH dans le sérum sanguin ont augmenté après le traitement par LH-RH et la réponse la plus marquée a été observée chez les vaches ayant des kystes folliculaires ovariens. Les concentrations en progestérone dans le sérum ont augmenté 6 heures après l'administration du LH-RH chez les animaux ayant du tissu lutéal fonctionnel dans les ovaires mais le niveau d'œstradiol et d'œstrone dans le sérum est resté inchangé. Les kystes folliculaires ovariens se sont lutéinisés après l'administration du LH-RH chez les vaches et les cycles œstriens ont été induits chez ces mêmes animaux. L'ovulation a été provoquée un jour après l'administration du LH-RH chez les vaches à deux semaines post-partum mais elle n'a pas été observée chez les génisses et les vaches en diœstrus ou chez les génisses en proœstrus traitées par LH-RH. Les résultats obtenus chez les bovins sont discutés en comparaison avec ceux obtenus chez les brebis, les truies et les juments après le traitement par l'hormone LH-RH.

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