The acute effects of oestradiol-17 β and synthetic LH-RH on plasma LH levels in freemartin heifers

N. F. Cunningham, N. Saba and C. D. Boarer

*Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food,
Weybridge, Surrey, KT15 3NB, U.K.*

Summary. Injection of oestradiol was followed by a surge of plasma LH within 24 h in only 7 of 12 freemartins. Elevations of plasma LH were less than those reported for normal non-cyclic heifers, but some freemartins showed a delayed, or more prolonged, LH response. Responsiveness to oestradiol was not related to degree of chimaerism or plasma androstenedione level, and most of the animals responded similarly in two trials carried out 4 months apart, during which time plasma androstenedione levels had more than doubled. Freemartins which showed an LH surge after oestradiol treatment released greater amounts of LH after the injection of LH-RH than did non-responders.

Introduction

The surge of plasma LH which follows the administration of oestradiol to normal cyclic heifers during the early stages of the oestrous cycle (Martin, Koligian, Ford & Stormshak, 1974) or to normal non-cyclic heifers (Saba, Symons, Cunningham & Boarer, 1976), has been shown to be diminished or absent in freemartin heifers (Saba *et al.*, 1976). Only 1 of 19 freemartins studied by Saba *et al.* (1976) responded with a normal LH surge, and a further 4 showed smaller increases in plasma LH level during the 24 h following the injection of oestradiol. The absence of a response in the remaining 14 animals may have been due to a lack of sensitivity of the freemartin hypothalamus to oestradiol. The rate of androstenedione production in freemartin heifers increases with age: in 1-year-old freemartins, plasma androstenedione levels were low, but by 2 years of age they had increased to values similar to those in normal heifers (Saba, Cunningham & Millar, 1975). To determine whether this change in steroid metabolism is accompanied by a change in the responsiveness to oestradiol, we have studied the effects of two injections of oestradiol given 4 months apart to freemartin heifers. We also present data on the release of LH following the administration of LH-RH to freemartins.

Materials and Methods

Experimental animals

Twelve Friesian heifers housed indoors were identified as freemartins by clinical examination (P. G. Millar, personal communication). They were similar to the undifferentiated female type of freemartin described by Saba *et al.* (1975) and were characterized by the absence of a developed cervix and uterine horns, and gonads which were small and difficult to identify by digital palpation *per rectum*. Only two of the animals (Nos 921 and 957) had palpable ovaries, estimated to measure about 0.1 $\times$ 0.5 cm. Cytogenetic studies (P. R. Wilkes, personal communication) showed that the proportion of XY leucocytes in the blood ranged from 12 to 92% (see Text-fig. 1).

Treatments

Oestradiol injection. Two trials were carried out—one in May when the animals were 17 months old, and the other 4 months later in September. Each animal was given an i.m. injection of 2 ml isotonic saline containing a fine suspension of 1 mg oestradiol-17 β (Sigma). Blood samples were

taken from the jugular vein into heparinized Vacutainers at 3-h intervals during the 24 h preceding the injection of oestradiol (control period), and during the 48 h following injection. Blood plasma was stored at -20°C until required for hormone assays.

LH-RH injection. This experiment was carried out in October, 4 weeks after the second oestradiol trial. The freemartins were treated i.m. with 500 μg synthetic LH-RH (Hoechst) in 2 ml isotonic saline. Samples of jugular blood were taken immediately before the injection, at 30-min intervals for the first 2 h, and at 1-h intervals for the next 5 h after the injection of LH-RH.

Radioimmunoassays

LH estimations were carried out on undiluted plasma by a solid-phase method utilizing PVC trays coated with antiserum (Boarer, Morris & Mackintosh, 1977). The ^{125}I -labelled hormone was a highly purified preparation of bovine LH (LER 1716-2) and unlabelled standards were NIH-LH-B8. Plasma androstenedione levels were determined by the charcoal-gelatin disk method (Saba, 1976) with an antiserum prepared against androstenedione-3-oxime-HSA (S 1557, No. 2; Dr G. E. Abraham).

Results and Discussion

Oestradiol injections

The plasma androstenedione and LH concentrations during the two trials, and the degree of chimaerism shown by each freemartin in the proportion of XY cells in the blood, are shown in Text-fig. 1.

Mean LH levels during the control period varied markedly between animals, but a comparison of the LH values for the first 24 h after oestradiol injection with those for the control period suggested that 5 freemartins (Nos 896, 897, 915, 938 and 951) responded with an oestradiol-induced surge of

Text-fig. 1. Plasma concentrations of LH and androstenedione in 12 freemartin heifers injected with 1 mg oestradiol in May and September. Each column represents the mean LH value of 8 samples taken at 3-h intervals during the 24 h preceding the injection of oestradiol (open columns) and during the periods 3–24 h (stippled columns) and 27–48 h (hatched columns) following oestradiol. The minimum and maximum LH concentrations during each period are indicated (●). The individual androstenedione concentrations (pg/ml plasma) at the start of the two trials are indicated above the columns.

plasma LH in both the May and September trials: LH levels > 10 ng/ml plasma were observed following the injection, and the peak values for each animal (11.1–47.9 ng LH/ml plasma) were higher than any found during the control period. These LH surges were reflected in an increase of mean LH levels after the injection of oestradiol in all 5 animals (Text-fig. 1). A further 2 freemartins showed an LH surge after oestradiol in only one of the trials: No. 901 responded in May with a peak LH concentration of 11.8 ng/ml plasma, but showed a slight fall in mean LH concentration. In September, all samples from this animal contained < 10 ng LH/ml plasma. No. 905 showed no response in May, but gave a clear-cut response in September (peak LH concentration, 13.0 ng/ml plasma). Only one freemartin (No. 896) responded to oestradiol with an LH surge approaching in magnitude that shown by normal heifers, with peak levels of 42.6 ng LH/ml (May) and 47.9 ng/ml (September) at 12–18 h. This compares with peak levels of 70–110 ng LH/ml between 15 and 21 h after a smaller dose of oestradiol (250 or 500 µg) in normal non-cyclic heifers (Saba *et al.*, 1976). The remaining 5 animals did not show an LH surge during the first 24 h after injection of oestradiol. In two (Nos 921 and 957), all plasma samples contained < 10 ng LH/ml, and in three (Nos 889, 916 and 949) higher plasma LH concentrations were observed during the control period than after the injection of oestradiol.

Plasma androstenedione concentrations measured 24 h before oestradiol injection in each trial showed marked individual variation and ranged from 11 to 78 pg/ml in May, and from 32 to 217 pg/ml in September (Text-fig. 1). The level increased in all animals during the 4 months between the two trials, resulting in a significantly higher ($P < 0.005$) mean androstenedione concentration in September (96.3 ± 15.64 (S.E.M.) pg/ml) than in May (39.0 ± 6.45 pg/ml). Despite the change in steroid metabolism suggested by these observations, individual freemartins showed similar LH responses to oestradiol in the two trials. The animal with the lowest percentage of XY leucocytes (No. 896) exhibited the greatest LH response in both trials, but there were no other correlations between the degree of chimaerism or plasma androstenedione level of individual animals and either basal LH level during the control period or responsiveness to oestradiol.

In our previous investigation on the response of freemartins to oestradiol (Saba *et al.*, 1976), classification of animals as positive responders or non-responders was based on the assumption that in freemartins, as in normal heifers, a response to oestradiol would occur during the first 24 h after injection. However, in the present experiments, two of the responders in the May trial (Nos 938 and 951) and three of those in September (Nos 915, 938 and 905) showed higher mean LH levels during the period 27–48 h than during the first 24 h following oestradiol (Text-fig. 1). Moreover, 7 animals in May and 8 in September showed higher mean and maximum LH concentrations during the second 24 h after oestradiol than during the control period. These observations suggest that, in some freemartins, the response to oestradiol may be delayed or more prolonged than in normal heifers. If the effect of oestradiol can extend into the second 24 h after injection, then the elevated plasma LH levels shown by two 'non-responders' (Nos 889 and 916) in both the May and September trials may represent a delayed response to oestradiol.

LH-RH injection

The effects of an i.m. injection of 500 µg LH-RH on plasma LH levels are presented in Table 1. The animals have been grouped according to the type of LH response to oestradiol shown in the September trial. There was a rapid rise of plasma LH concentration in all of the freemartins and peak LH levels were observed in the 30-min sample from 10 animals, and in the 1 h sample from two (Nos 896 and 916). This was followed by a fall in LH concentrations, which had returned to basal values by 5 h after LH-RH. The LH response was more rapid than that generally reported for normal heifers, in which peak LH levels occurred 2–2½ h after the injection of LH-RH (Mauer & Rippel, 1972; Kaltenbach *et al.*, 1974; Dermody, Reel, Beck & Coppock, 1976). However, Zolman, Convey & Britt (1974) observed peak LH concentrations 30 min after LH-RH in cyclic heifers which were younger (12–16 months) than the freemartins used in the present experiments (21 months). An early LH response to LH-RH is also characteristic of immature calves (Symons, 1976) and castrated sheep (Rippel, Moyer, Johnson & Mauer, 1974; Saba, Symons & Drane, 1974).

Table 1. The effect of an injection of 500 µg LH-RH on plasma LH concentrations in freemartin heifers

LH response to oestradiol*	Animal no.	LH conc. (ng/ml plasma)		
		Preinjection	Maximum increment after LH-RH	Area under LH-RH response curve†
Positive	896	10.4	49.7	105.4
	897	2.6	36.1	61.7
	905	3.6	38.6	52.4
	915	5.0	42.3	80.6
	938	10.0	30.8	64.6
	951	7.4	29.7	64.6
	Mean ± S.E.M.		37.9 ± 3.05	71.6 ± 7.72
No response	901	1.8	24.5	41.0
	921	3.3	22.1	33.9
	949	5.0	16.5	31.1
	957	1.3	15.1	23.5
	Mean ± S.E.M.		19.6 ± 2.24	32.4 ± 3.62
Delayed	889	5.6	21.6	50.5
	916	6.5	35.5	84.7

* Based on results of September trial.

† Area (ng/ml × h) above basal level for the first 5 h after the LH-RH injection.

The positive-response animals showed a significantly greater release of LH than the non-responding animals in terms of both maximum increments in plasma LH ($P < 0.005$) and areas under the LH-RH response curves ($P < 0.005$). Of the 2 freemartins which showed a delayed response to oestradiol, one (No. 889) showed an increment of plasma LH after LH-RH within the range shown by the no-response group, and the other (No. 916) showed an increment similar to that in the positive-response group. However, the areas under the response curves of both animals were comparable to those of the positive-response group.

The results of the present experiments confirm our previous observations (Saba *et al.*, 1976) that only some freemartins display an LH surge after oestradiol treatment, and that the responses are generally less marked than those shown by normal heifers. However, the present observations that the pituitaries of freemartins responding to oestradiol discharge significantly greater amounts of LH after the injection of LH-RH than do those of freemartins that do not respond to oestradiol would suggest that the difference between responders and non-responders may involve pituitary dysfunction.

We wish to thank Hoechst Pharmaceuticals for the gift of synthetic LH-RH and the National Institute of Arthritis, Metabolism and Digestive Diseases, U.S.A. for bovine LH preparations. We are also indebted to Mr R. Mason and Mrs V. Mackintosh for skilled technical assistance.

References

- BOARER, C.D.H., MORRIS, C.R. & MACKINTOSH, V. (1977) Radioimmunoassay of ovine luteinizing hormone in PVC disposable trays using a cutting aid. *Med. Lab. Sci.* **34**, 155–162.
- DERMODY, W.C., REEL, J.R., BECK, C.C. & COPPOCK, R.W. (1976) Serum luteinizing hormone concentrations in dairy heifers after intravenous and intra-uterine administration of luteinizing hormone-releasing hormone. *Vet. Med. & Small Anim. Clin.* **71**, 419–422.
- KALTENBACH, C.C., DUNN, T.G., KISER, T.E., CORAH, L.R., AKBAR, A.M. & NISWENDER, G.D. (1974) Release of FSH and LH in beef heifers by synthetic gonadotrophin releasing hormone. *J. Anim. Sci.* **38**, 357–362.
- MARTIN, S.K., KOLIGIAN, K.B., FORD, S.P. & STORMSHAK, F. (1974) Estradiol-induced release of LH in beef heifers. *Proc., West. Sectn, Am. Soc. Anim. Sci.* **25**, 267–268.
- MAUER, R.E. & RIPPPEL, R.H. (1972) Response of cattle

- to synthetic gonadotropin releasing hormone. *J. Anim. Sci.* **35**, 249.
- RIPPEL, R.H., MOYER, R.H., JOHNSON, E.S. & MAUER, R.E. (1974) Response of the ewe to synthetic gonadotropin releasing hormone. *J. Anim. Sci.* **38**, 605-612.
- SABA, N. (1976) Adsorption of free steroids with charcoal-gelatin disks in radioimmunoassay. *J. Endocr.* **70**, 141-146.
- SABA, N., CUNNINGHAM, N.F. & MILLAR, P.G. (1975) Plasma progesterone, androstenedione and testosterone concentrations in freemartin heifers. *J. Reprod. Fert.* **45**, 37-45.
- SABA, N., SYMONS, A.M., CUNNINGHAM, N.F. & BOARER, C.D. (1976) The acute effect of oestrogen injection on plasma LH in freemartin heifers. *J. Reprod. Fert.* **48**, 317-321.
- SABA, N., SYMONS, A.M. & DRANE, H.M. (1974) The effects of feeding white clover pellets and red clover hay on teat length, plasma gonadotrophins and pituitary function in wethers. *J. agric. Sci., Camb.* **82**, 357-361.
- SYMONS, A.M. (1976) The LH response of pregnant cows and their calves to synthetic LH-RH. *J. Reprod. Fert.* **46**, 235-237.
- ZOLMAN, J., CONVEY, E.M. & BRITT, J.H. (1974) Relationships between the luteinizing hormone response to gonadotropin releasing hormone and endogenous steroids. *J. Anim. Sci.* **39**, 355-359.

Received 7 September 1976