J. Reprod. Fert. (1984) **71**, 525–531 *Printed in Great Britain*

LH concentrations in two cattle with XY gonadal dysgenesis

K. L. Macmillan, E. D. Fielden*, K. P. McNatty† and H. V. Henderson

*Ruakura Animal Research Station, Private Bag, Hamilton; *Massey University, Private Bag, Palmerston North; and †Wallaceville Animal Research Station, Upper Hutt, New Zealand*

Summary. Two animals with XY gonadal dysgenesis both had a reproductive tract similar in size to that found in sexually immature heifers, but neither had normal testicular or ovarian tissue. All cells examined in both animals contained XY chromosomes and spinal cord neurones were sex chromatin negative. Basal LH concentrations averaged 3·1 ng/ml in Animal 1 and 2·4 ng/ml in Animal 2 but increased within 12 h of injecting oestradiol to peak concentrations of 125 and 11 ng/ml respectively. Animal 1 displayed a distinct pulsatile LH release pattern with a highly repeatable decline phase at each pulse. A GnRH injection produced a rapid rise in plasma LH in both animals, sustained in Animal 1 at >500 ng/ml for more than 2 h. Each animal displayed behavioural symptoms of oestrus within 12 h of being injected with 3 mg oestradiol benzoate and was repeatedly served by a bull.

These studies indicated that both animals differed from freemartins and had some hypothalamic and pituitary response patterns resembling those reported for female cattle.

Introduction

The most frequent form of intersexuality in cattle is freemartinism. It occurs in heterosexual twin conceptions as a sequel to the fusing of the chorioallantois (Short, 1970; Jost, Vigier, Prepin & Perchellett, 1973), but the degree of abnormal development varies (Jost, Vigier & Prepin, 1972). Intersexuality is rare in cattle born as singletons, especially when associated with a normal chromosomal complement. Nes (1966), McFeely, Hare & Biggers (1967), Long & David (1981) and Gustavsson, Settergren, Gustafsson & Larsson (1981) have described a limited number of cases of testicular feminization with pure XY sex chromosomal background. In every case, external features were mainly female but the internal reproductive tract was predominantly masculine. XY gonadal dysgenesis in cattle is associated with substantial development of the cervix, uterus and vagina (Henricson & Akesson, 1967; Chapman, Bruère & Jaine, 1978; Sharma, Vijaykumar, Khar, Verma & Nigam, 1980; Gustavsson *et al.*, 1981).

Plasma steroid hormones and luteinizing hormone (LH) have been measured in freemartins (Saba, Cunningham & Millar, 1975; Saba, Symons, Cunningham & Boarer, 1976). Although concentrations vary between animals, almost all acyclic freemartins have lost the female responsiveness to release LH after an oestrogen injection (Saba *et al.*, 1976). A similar reduction in responsiveness has been reported in men with testicular feminization (Aona, Miyake, Kinugasa, Kurachi & Matsumoto, 1978) and in ewes whose dams were injected with testosterone during pregnancy (Clarke & Scaramuzzi, 1978). In contrast, LH is released as a sequel to an oestrogen injection in humans with XY gonadal dysgenesis (Leydendecker, Wardlaw, Leffek & Nocke,

1971). Comparable studies have not been made in cattle with testicular feminization or XY gonadal dysgenesis. In this report, two acyclic animals were karyotyped, identified as potential cases of XY gonadal dysgenesis and were studied to determine whether the animals exhibited behaviour patterns and hormone responses similar to those of male or female cattle.

Materials and Methods

Animals and karyotyping. The first animal (Animal 1) was a 3/4 Charolais : 1/4 Angus born in November 1973 and initially presented in September 1976 because of failure to display oestrus. The procedure resulting in the diagnosis of XY gonadal dysgenesis has been described by Chapman *et al.* (1978). In November 1976, when karyotyping had been completed, the animal was trained to continuous tethering and frequent handling. A jugular cannula was inserted during this training period and was then used during a 3-day blood sampling programme.

The second animal (Animal 2) was a Simmental imported from the United Kingdom and presented as acyclic in February 1980, when estimated to be 4 or 5 years of age. A similar karyotyping, training and preparative procedure was followed with this animal.

Blood sampling. On each collection day, 20-ml blood samples were obtained from the jugular cannula at 15-min (Animal 1) or 20-min (Animal 2) intervals. For Animal 1, the sampling periods were from 08 : 00 to 16 : 30 h on 3 consecutive days. For Animal 2, the first day's sampling lasted 24 h and was followed by a 1-day break, a 08 : 00–18 : 00 sampling period, then a second 1-day break and finally a 3rd sampling period (08 : 00–18 : 00 h). From a knowledge of ovarian blood flow (Ford & Chenault, 1981; Wise, Caton, Thatcher, Lehrer & Fields, 1982), haematocrit and the ovarian venous concentrations of oestradiol around oestrus (1–7 ng/ml; K. P. McNatty, unpublished data) it can be calculated that the secretion rate of oestradiol from a bovine ovary containing a dominant oestrogen-secreting follicle may vary from 3 to 300 μg/day. Therefore, to test the ability of oestradiol to stimulate the release of LH we chose an oestradiol dose of 125 μg for Animal 1 and 50 μg for Animal 2 as representing amounts of oestradiol which might commonly be experienced by a normal cow during the follicular phase of the oestrous cycle.

For neither animal was the first day of sampling associated with any treatment. At 8 h before sampling began on the second day, each animal was injected intramuscularly with 125 μg oestradiol-17β in 0·154 M-NaCl (Animal 1) or 50 μg oestradiol benzoate in 0·154 M-NaCl (Animal 2). On the 3rd day of sampling, each animal was injected intramuscularly with 100 μg (Animal 1) or 200 μg (Animal 2) of synthetic GnRH (Luliberin, Pierce Chem. Co., U.S.A.) 3 min after sampling at 08 : 00 h.

Each sample was collected into a heparinized tube, cooled to 4°C and then centrifuged. Plasma was stored at −20°C.

LH assays. The assay has been described by McNatty, Gibb, Dobson, Thurley & Findlay (1981). The antibody was raised in a rabbit using NIH-LH-S11 as antigen and was used at an initial dilution of 1 : 50 000. This LH was also used as the iodinated tracer in assays with NIH-LH-B9 as the standard. The antiserum cross-reacted with NIH-P-B5 (0·12%), bovine TSH (Schwarz–Mann) (<0·01%), NIH-GH-S11 (0·4%), NIH-FSH-S10 (0·3%) and bovine serum albumin, fraction V (<0·01%). The minimum detection limit was 0·3 ng/ml. The intra- and inter-assay coefficients of variation were 10% and 15% respectively.

Sexual behaviour. At least 2 weeks after the blood sampling programme and removal of the cannula, each animal was injected intramuscularly with 3 mg oestradiol benzoate in oil and returned to a group of animals which included several normally cyclic cows and a sexually experienced bull. The group was observed periodically during the ensuing 48 h for behavioural activity associated with oestrus.

Statistical analyses. Geometric means were used for comparing treatment effects between Day 1 and Days 2 or 3. Regression analyses were made on the decline phases of each LH pulse for Animal 1 on Days 1 and 2 using log-transformed data.

Results

Karyotyping and clinical diagnosis

Initial anatomical and cytogenetic findings for Animal 1 have been described by Chapman *et al.* (1978). The animal had a karyotype of 60XY in all of the 459 cells from 6 blood or tissue samples. None of 100 cells examined from the cerebellum and spinal cord contained a sex chromatin body. The vulva and vagina were morphologically normal, the cervix had a double external os and the uterus was infantile. A left 'gonad' was present but no right gonad was found. Tissue from the left gonad was examined histologically on our behalf by Dr Hannah Peters. No germ cells were detected but the gonadal stroma contained ducts resembling rete tubules. Tumour tissue, most probably associated with gonadoblastoma, was present throughout the specimens. A cystic dilatation of the right oviduct was found to be a hydrosalpinx. There was no histological evidence of neoplasia in the tubular reproductive tract. Chapman *et al.* (1978) concluded that this animal was displaying clinical features of XY gonadal dysgenesis.

With Animal 2, the 30 clear metaphase spreads after leucocyte culture and 21 cells examined from the fibroblast cultures were all 60XY. All 100 neurones examined from sections of the spinal cord were chromatin-negative. All the cytogenetic data indicated that this animal had an XY karyotype. The udder and teats were small; the gross appearance of the tubular reproductive tract was normal, except that it was small in size, and the two gonads were poorly developed. Measurements taken at necropsy were: external cervical urethral opening to external cervical os—23 cm; external to internal cervical os—8 cm; internal cervical os to cranial tips of uterine horns—16·5 cm; right and left 'ovaries'—2·6 × 0·8 × 0·7 and 2·8 × 1·0 × 0·7 cm respectively. The cervical canal and lumen of the uterine body and horns were normal in appearance and patent.

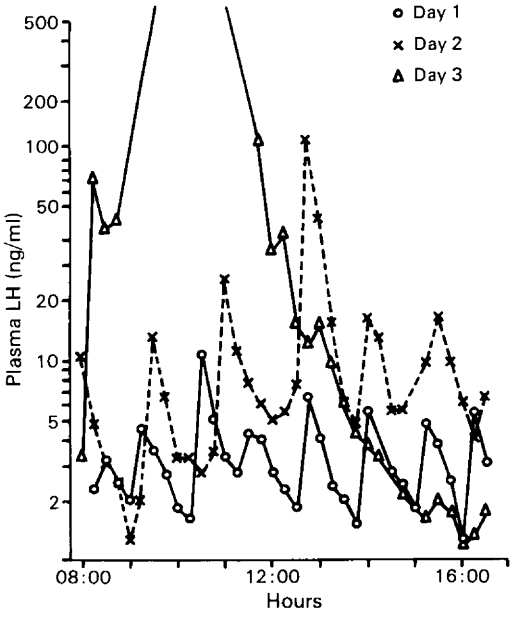
The ovarian cortex was hypoplastic and only occasional primordial and primary follicles were present. Cystic structures containing periodic acid–Schiff positive material, and 50–150 μm in diameter, were scattered throughout the cortical tissue. The medulla appeared histologically normal. The superficial epithelium of the uterus was normal, but duct development was reduced. Most of these ducts were simple tubular structures without coiling or branching.

LH profiles

All samples from both animals contained LH concentrations above the minimum detectable level. An injection of oestradiol (Day 2) or GnRH (Day 3) increased mean concentrations of plasma LH in both animals ($P < 0·01$; Table 1). With Animal 1, a distinct pulsatile pattern in LH release

Table 1. Mean and maximum plasma LH concentrations (ng/ml) in blood samples from Animals 1 and 2 with XY gonadal dysgenesis after an injection of oestradiol (Day 2) or GnRH (Day 3)

	Animal 1			Animal 2		
LH	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Mean	3·1	7·4	>28·6	2·4	5·1	6·9
s.e.m.	0·2	0·5	>1·7	0·2	0·2	0·5
Maximum	10·5	125·0	>500·0	3·5	11·2	75·0



Text-fig. 1. Plasma LH concentrations with 15-min sampling before treatment (Day 1) and after an injection of oestradiol (Day 2) or GnRH (Day 3) in Animal 1.

was observed on Days 1 and 2 (Text-fig. 1), but the interval between peaks increased from 1·11 ± 0·07 h (s.e.m.) on Day 1 to 1·44 ± 0·09 h on Day 2 ($P < 0·05$). The maximum LH concentration on Day 2 (125 ng/ml) was measured in the sample collected 12·75 h after the oestrogen injection (Text-fig. 1). The GnRH injection produced a rapid rise in plasma LH. The 11 samples collected from 1 to 3·5 h after GnRH administration had concentrations of >500 ng/ml (Text-fig. 1).

There were 13 LH pulses from Animal 1 on Days 1 and 2 which were subjected to decline-phase regression analyses. The equation describing these decline phases was:

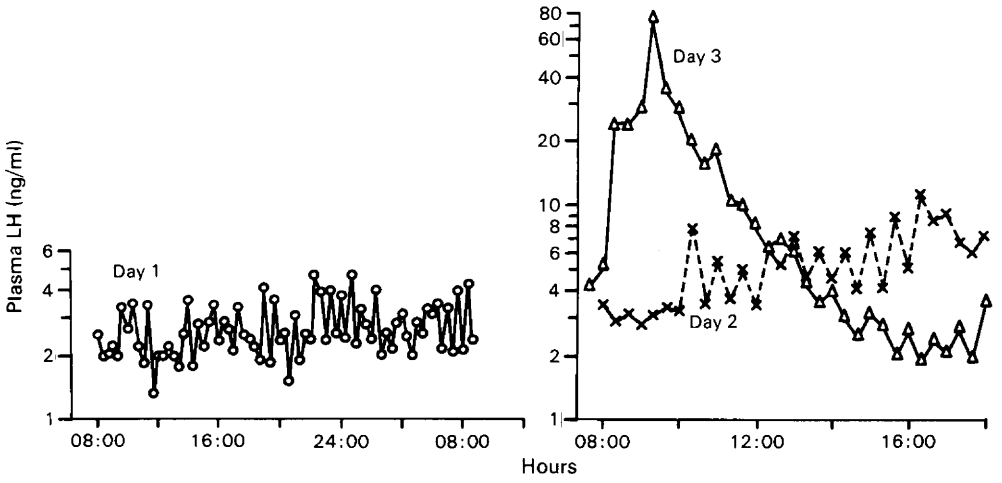
$$\log_e LH_{i+1} = 0·764 \log_e LH_i \text{ ng/ml } (R^2 = 0·95) \quad (\pm 0·013)$$

where LH_i = concentration in sample at time i on a decline phase;
and LH_{i+1} = concentration in sample taken 15 min later on the same decline phase.

A 20-min sampling interval did not allow precise definition of a pulsing pattern of LH release in Animal 2 (Text-fig. 2). On Day 1 the mean LH concentration for the 24-h sampling period was 2·5 ± 0·1 ng/ml, varying from 2·4 ± 0·2 ng/ml between 08:00 and 18:00 h (Table 1) to 2·7 ± 0·2 ng/ml between 18:20 and 07:40 h (Text-fig. 2). The maximum LH concentrations on Days 2 and 3 of 11·2 and 75 ng/ml respectively were measured in samples collected 16·3 and 1 h after an oestradiol or GnRH injection (Text-fig. 2).

Sexual behaviour

At 6 h after an injection of 3 mg oestradiol benzoate, a bull attempted to mount Animal 1. Within 24 h it had been intensively ridden by a non-oestrous cow and served several times by the bull. Semen was swabbed from the vagina. This animal had not been observed previously to stand when mounted or to attempt to mount herdmates of either sex. A similar behavioural sequence was observed with Animal 2 although the interval from treatment until the animal aroused the interest of a bull was 12 h. Involvement in sexual activity waned within 48 h and did not recur in either animal.



Text-fig. 2. Plasma LH concentrations with 20-min sampling before treatment (Day 1) and after an injection of oestradiol (Day 2) or GnRH (Day 3) in Animal 2.

Discussion

The changes in LH concentration and sexual behaviour occurring after selected oestradiol treatments in both animals were more typical of those associated with cows than with bulls or steers. Positive oestradiol feedback responses in LH occurred within 17 h of injection (Text-figs 1 & 2) even though comparatively low doses were injected.

The 15-min blood sampling interval used with Animal 1 permitted the pulsatile pattern of LH release to be clearly defined (Text-fig. 1). The pulse intervals of 1·11 (Day 1) to 1·44 h (Day 2) were similar to those reported by Schallenberger & Peterson (1982) for recently ovariectomized cows, although the amplitude of each pulse in those cows was only 1·5 ng/ml compared to 4·1 ng/ml with Animal 1 on Day 1 (Text-fig. 1). Rahe, Owens, Fleeger, Newton & Harms (1980) found a similar pulse frequency in cows sampled during early pro-oestrus or late metoestrus.

The consistent pattern of the decline phase after each LH pulse in Animal 1 on Days 1 and 2 shows that the metabolic clearance rate of LH (as reflected by plasma LH concentrations) followed a highly repeatable decay curve. The equation for this curve can be used to derive an estimate of the half-life ($t_{1/2}$) of LH:

$$t_{1/2} = 15\left[\log_e\left(1 - \frac{\log_e 2}{\log_e \mathrm{LH}}/\log_e{}^{0\cdot764}\right)\right] \text{ min}$$

Use of this equation shows that, if the LH concentration was 100 ng/ml, then $t_{1/2}$ would be 9·1 min. Comparable values of $t_{1/2}$ for LH concentrations of 50, 10 and 5 ng/ml would be 10·9, 20·0 and 31·4 min respectively. The final dramatic decline in LH on Day 3 after a GnRH injection (Text-fig. 1) could reflect the fact that the calculated decline-phase equation would allow the LH concentration to fall from a peak height of 500 to 7·2 ng/ml in 64 min in the absence of any further LH release. The relevance of this LH decline pattern to normal animals remains to be elucidated.

The mean plasma concentrations of LH for both animals on Day 1 (3·1 and 2·4 ng/ml) were higher than those reported for cows in dioestrus (Schams, Schallenberger, Hoffmann & Karg, 1977; Rahe *et al.*, 1980) but less than those in most freemartins (Cunningham, Saba & Boarer, 1977). However, these comparisons do not take account of differences in LH pulse frequency or amplitude. Both animals experienced rapid and sustained increases in plasma LH after an injection of GnRH (Text-figs 1 & 2). A plasma LH concentration of > 500 ng/ml was maintained for at least 2·5 h by Animal 1, indicating that this animal had a pituitary with substantial reserves of LH. It is

unlikely that an injection of 125 μg oestradiol 32 h before the GnRH would have increased pituitary sensitivity to the latter hormone (Zolman, Convey & Britt, 1974; Beck & Convey, 1977). The maximum LH concentrations after the GnRH injection in both animals were substantially higher than those reported after injecting pregnant cows (Symons, 1976) or freemartins with 500 μg GnRH (Cunningham *et al.*, 1977), but these differences may reflect variation in GnRH potency.

Mean (±s.e.m.) steroid hormone concentrations in samples from Animal 1 on Day 1 were: 0·65 ± 0·02 ng progesterone/ml, 173 ± 5 pg androstenedione/ml and 247 ± 2 pg testosterone/ml. Comparable means for Animal 2 were 0·18 ± 0·02 ng/ml, 100 ± 2 pg/ml and 63 ± 1 pg/ml respectively (K. P. McNatty, personal communication). Oestradiol-17β was less than the detectable limit for the assays in most samples from both animals. Only minor changes occurred in average steroid hormone concentrations in either animal on Days 2 and 3 when LH concentrations were elevated. These low average concentrations and the lack of trophic responses indicate that neither animal possessed gonadal tissue which could normally be stimulated by LH during the sampling periods. Nonetheless, the lack of an obvious pulsatile pattern of LH release in Animal 2 (Text-fig. 2) may reflect steroid hormonal influence on the hypothalamus or pituitary.

The dose of 3 mg oestradiol that produced standing oestrus in both animals, culminating in repeated service by a bull, will not produce behavioural oestrus in steers, bulls or cows during dioestrus (Greene, Mogil & Foote, 1978). Although Greene *et al.* (1978) concluded that the neural centres responsible for sexual behaviour in freemartins had not been affected by androgens, standing when mounted was not observed in untreated freemartins and infrequently observed in hormonally treated contemporaries.

Both Animals 1 and 2 developed a female tubular reproductive tract in the absence of effects associated with normal fetal testicular tissue (Wilson, Griffin & George, 1980). It is probable that any influence from fetal ovarian tissue was also lacking, and that neither animal had ever experienced prolonged effects from any potent sex steroid at the physiologically normal concentrations found in male or female cattle. This could mean that each of them had a neurohypophysial system which had not been substantially modified or developed by oestrogen or testosterone (Desjardins, 1981).

We thank Dr Hannah Peters for histological advice, Marion Gibb and Linda Kieboom for steroid assays, Professor A. N. Bruère for cytogenetic examinations, Dr A. C. Johnstone for histological examinations, and the NIH Pituitary Agency, U.S.A., for supplies of purified pituitary hormones.

References

Aona, T., Miyake, A., Kinugasa, T., Kurachi, K. & Matsumoto, K. (1978) Absence of positive feedback effect of oestrogen on LH release in patients with testicular feminization syndrome. *Acta endocr., Copenh.* **87**, 259–267.

Beck, T.W. & Convey, E.M. (1977) Estradiol control of serum luteinizing hormone concentrations in the bovine. *J. Anim. Sci.* **45**, 1096–1101.

Chapman, H.M., Bruère, A.N. & Jaine, P.M. (1978) XY gonadal dysgenesis in a Charolais heifer. *Anim. Reprod. Sci.* **1**, 9–18.

Clarke, I.J. & Scaramuzzi, R.J. (1978) Sexual behaviour and LH secretion in spayed androgenized ewes after a single injection of testosterone or oestradiol-17β. *J. Reprod. Fert.* **52**, 313–320.

Cunningham, N.F., Saba, N. & Boarer, C.D. (1977) The acute effects of oestradiol-17β and synthetic LH-RH on plasma LH levels in freemartin heifers. *J. Reprod. Fert.* **51**, 29–33.

Desjardins, C. (1981) Endocrine signaling and male reproduction. *Biol. Reprod.* **24**, 1–21.

Ford, S.P. & Chenault, J.R. (1981) Blood flow to the corpus luteum-bearing ovary and ipsilateral uterine horn of cows during the oestrous cycle and early pregnancy. *J. Reprod. Fert.* **62**, 555–562.

Greene, W.A., Mogil, L. & Foote, R.H. (1978) Behavioural characteristics of freemartins administered estradiol, estrone, testosterone, and dihydrotestosterone. *Horm. Behav.* **10**, 71–84.

Gustavsson, I., Settergren, I., Gustafsson, H. & Larsson, K. (1981) Testicular feminization and XY gonadal dysgenesis in Swedish Red and White cattle. *J. Anim. Sci.* **53**, Suppl. 1, 160, Abstr.

Henricson, B. & Akesson, A. (1967) Two heifers with gonadal dysgenesis and the sex chromosomal constitution XY. *Acta vet. scand.* **8**, 262–272.

Jost, A., Vigier, B. & Prepin, J. (1972) Freemartins in cattle: the first steps of sexual organogenesis. *J. Reprod. Fert.* **29**, 349–379.

Jost, A., Vigier, B., Prepin, J. & Perchellet, J.P. (1973) Studies of sex differentiation in mammals. *Recent Prog. Horm. Res.* **29**, 1–35.

Leydendecker, G., Wardlaw, S., Leffek, B. & Nocke, W. (1971) Studies on the function of the hypothalamic sexual centre in the human: presence of a cyclic centre in a genetic male. *Acta endocr., Copenh., Suppl.* **155**, 36, Abstr.

Long, S.E. & David, J.S.E. (1981) Testicular feminisation in an Ayrshire cow. *Vet. Rec.* **109**, 116–118.

McFeely, R.A., Hare, W.C.D. & Biggers, J.D. (1967) Chromosome studies in 14 cases of intersex in domestic mammals. *Cytogenetics* **6**, 242–253.

McNatty, K.P., Gibb, M., Dobson, C., Thurley, D.C. & Findlay, J.K. (1981) Changes in the concentration of gonadotrophic steroidal hormones in the antral fluid of ovarian follicles throughout the oestrous cycle of the sheep. *Aust. J. biol. Sci.* **34**, 67–80.

Nes, N. (1966) Testikulaer feminisering hos storfe. *Nord. VetMed.* **18**, 19–29.

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

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.

Schallenberger, E. & Peterson, A.J. (1982) Effect of ovariectomy on tonic gonadotrophin secretion in cyclic and post-partum dairy cows. *J. Reprod. Fert.* **64**, 47–52.

Schams, D., Schallenberger, E., Hoffmann, B. & Karg, H. (1977) The oestrous cycle of the cow: hormonal parameters and time relationships concerning oestrus, ovulation and electrical resistance of the vaginal mucus. *Acta endocr., Copenh.* **86**, 180–192.

Sharma, A.K., Vijaykumar, N.K., Khar, S.K., Verma, S.K. & Nigam, J.M. (1980) XY gonadal dysgenesis in a heifer. *Vet. Rec.* **107**, 328–330.

Short, R.V. (1970) The bovine freemartin: a new look at an old problem. *Phil. Trans. R. Soc. B* **259**, 141–147.

Symons, A.M. (1976) The LH response of pregnant cows and their calves to synthetic LH-RH. *J. Reprod. Fert.* **46**, 235–237.

Wilson, J.D., Griffin, J.E. & George, F.W. (1980) Sexual differentiation: early hormone synthesis and action. *Biol. Reprod.* **22**, 9–17.

Wise, T.H., Caton, D., Thatcher, W.W., Lehrer, A.R. & Fields, M.J. (1982) Androstenedione, dehydroepiandrosterone and testosterone on ovarian vein plasma and androstenedione in peripheral arterial plasma during the bovine oestrous cycle. *J. Reprod. Fert.* **66**, 513–518.

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 1 November 1983