Endocrine patterns associated with puberty in male and female cattle

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Summary. In four studies secretion patterns of LH, FSH, prolactin, testosterone and progesterone were measured in male and female cattle to determine endocrine changes associated with sexual maturation. Two periods of increasing gonado-trophin secretion were observed, the second one coinciding with puberty. A short luteal phase of 8–12 days precedes the first oestrus at 10–11 or 14 months of age. The testosterone values of the bulls increased with age from 5–6 months. Prolactin levels in the bulls increased at the time of the first testosterone rise.

A frequency of about 1–2 LH and FSH pulses/8 h occurred at 1, 2, 5 and 10 months of age. The pituitary response to GnRH (1 µg/kg i.v.) was tested in 2 male and 2 female calves at monthly intervals. LH and FSH were released at all ages but a reduced FSH response occurred in both sexes after the 5th month. A small testosterone release was observed as early as 1 month after birth in males similar to those observed after endogenous LH pulses.

We conclude that the initiation of puberty in both sexes is controlled by the same neuroendocrine mechanisms. The pituitary gland, ovaries and testes are already able to respond to specific stimuli long before puberty, and they may also be involved indirectly due to changes in the feedback system modulating gonadotrophin secretion.

Introduction

The endocrine mechanisms which govern sexual maturation in cattle are not yet fully understood. In order to elucidate hormonal changes and interrelationships occurring during puberty, the concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, progesterone and testosterone were measured in male and female animals at different ages. The present paper tries to summarize our results, part of which have been reported elsewhere (Schams & Butz, 1972; Karg *et al.*, 1976). Contributions of other groups are referred to within the discussion.

Materials and Methods

Animals

Experiments were carried out at the research station in Weihenstephan with animals of the local Brown Swiss breed which were kept under identical management conditions. The calves were separated from their mothers at birth and housed indoors. Included in the present study are results of a parallel experiment which was carried out with British Friesian bulls in Nairobi, Kenya.

Experimental design

Experiment 1. Five female animals (born in May–June) were bled (20 ml) 3 times/week from birth up to 12 (N = 4) or 14 (N = 1) months of age. Bleedings were carried out every 6 h from the first detection of heat to the completion of the first oestrous cycle. First oestrous symptoms were noticed at 10 (N = 2), 11 (N = 2) and 14 (N = 1) months of age.

Experiment 2. Four bulls (born in May, July, October and November) were bled (20 ml) 3 times/week from birth up to 18 months of age. Puberty of male animals of this breed occurs at an age of 8–10 months. Four British Friesian bulls were examined from 4 to 18 months of age; these were kept under an equatorial climate at Nairobi, Kenya. These animals were bled (20 ml) 3 times/week. Puberty of males of this breed and kept indoors and fed poor quality hay and grass takes place at 14–16 months of age.

Experiment 3. The frequency of pulsatile gonadotrophin secretion was measured in 2 males and 2 females at 1, 2, 5 and 10 months of age. The animals were bled (10 ml) at 15-min intervals for 8 h by means of an indwelling jugular catheter.

Experiment 4. The response of the pituitary gland to synthetic gonadotrophin-releasing hormone (GnRH) was examined in 7 animals: 2 males and 2 females received 1 µg GnRH/kg i.v., at about monthly intervals from birth up to 9 months. The remaining animals received 250 µg GnRH i.v. at 1, 2, 3 and 10 months of age. Blood (10 ml) was collected from the jugular vein at 15-min intervals for 3 h, and for a further 2–3 h samples were taken every 30–60 min. The blood was cooled to 4°C and centrifuged within 1 h. Plasma samples were kept at −20°C until assayed.

Hormone determinations

Prolactin, LH, FSH, testosterone and progesterone were measured by the radioimmuno-assays described by Schams & Karg (1969a, b), Schams & Schallenberger (1976), Karg *et al.* (1976) and Hoffmann, Kyrein & Ender (1973). Sensitivities of the assays were 0·3 ng/ml for prolactin, 0·25 ng/ml for LH, 20 ng/ml for FSH, 20 pg/ml for testosterone and 30 pg/ml for progesterone. The following bovine pituitary preparations were used as reference standards for prolactin (NIH-P-B3, 24·1 i.u./mg), for FSH (NIH-FSH-B1, 0·49 × NIH-FSH-S1) and for LH (III-17-BP, 0·9 × NIH-LH-S1). The protein hormone assays are highly specific and do not show cross-reactions: endogenous oestrogens, androgens and corticoids exhibit no cross-reaction with the antiserum raised against 11α-hydroxyprogesterone–BSA. The antiserum raised against testosterone-11-hemisuccinate–BSA had a negligible cross-reaction with androstanolone and androstendione. Average inter-assay variation was 10–15%.

Results

Gonadotrophin and progesterone levels in female calves (Exp. 1)

The basal gonadotrophin levels exhibited a biphasic profile (Text-fig. 1). The mean LH and FSH values increased from birth to 3 months and then decreased reaching a nadir at 5 and 6 months. The values then increased again to reach a second peak at 9 months. A low progesterone level (<0·1 ng/ml) was present from 1 to 9 months of age (Text-fig. 1). An increase in progesterone production occurred in 4 out of the 5 animals at 10 months. The first oestrus was detected in these 4 heifers between 10 and 11 months. The 5th animal showed the first signs of oestrus at 14 months; this was not included in Text-fig. 1. Elevated progesterone concentrations, ranging up to 0·9 ± 0·1 ng/ml (±s.e.m.) were observed for 8–12 days before the first oestrus (Text-fig. 2a). The first oestrous cycle of the 4 heifers averaged 19 days (18–21). The 5th heifer exhibited a progesterone secretion pattern which resembled that of a normal corpus luteum

during an 18-day period before the first detected oestrus (Text-fig. 2b). Prior to this rise values were elevated for 8 days, but only to a maximal value of 1·2 ng/ml. The 4 animals included in Text-fig. 2(a) had pronounced preovulatory LH and FSH surges before and after the first oestrous cycles. Ovulations were confirmed by rectal palpation.
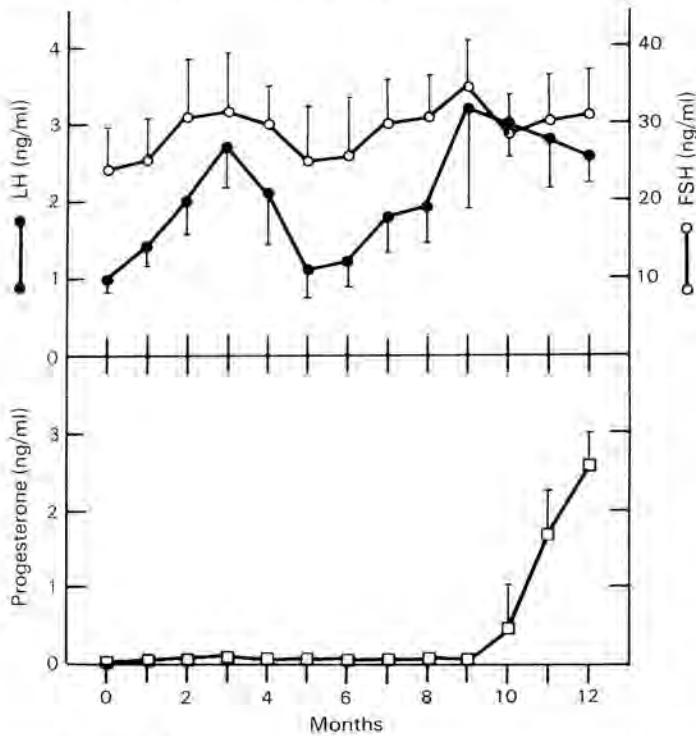

Text-fig. 1. Plasma LH, FSH and progesterone concentrations ($\pm$s.e.m.) of 4 female calves from birth to 12 months of age. Each point represents the mean monthly value of 52 samples (approximately 13 for each animal).
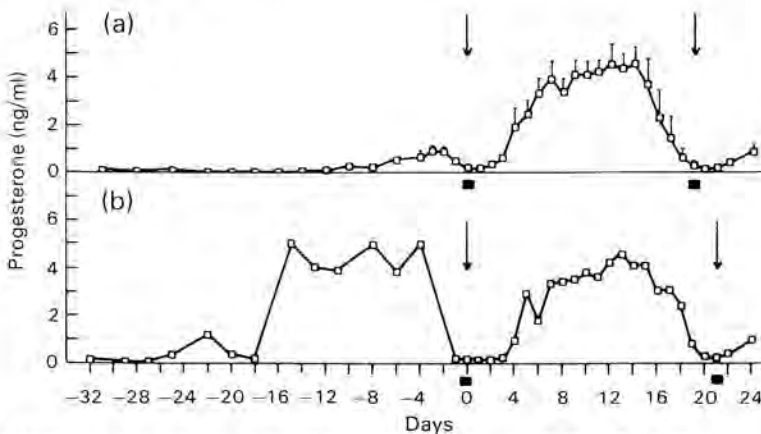
Text-fig. 2. Mean $\pm$s.e.m. plasma progesterone levels before and after the first oestrus (at 303 $\pm$ 11 days of age ($\pm$s.e.m.)) in (a) 4 heifers and (b) one heifer with first oestrus at 414 days of age. Progesterone concentrations were determined from daily samples. The arrows indicate the time of the preovulatory LH surges; ■ indicates oestrus.

Gonadotrophin, prolactin and testosterone levels in male calves (Exp. 2)

Basal gonadotrophin secretion increased from birth to 3 months of age and decreased thereafter to reach a nadir at 7 or 8 months (see Text-fig. 3a). The following more pronounced FSH rise had a duration of 7 months whereas the minor LH increase lasted for only 4 months. Testosterone concentrations were higher at birth than during the next 4 months. From 5 months the levels increased progressively except during Months 8, 12 and 13 (Text-fig. 3a). Average prolactin values decreased from birth to 5 months of age. A sharp increase was observed at 6 to 7 months; prolactin values decreased slowly thereafter (Text-fig. 3a).
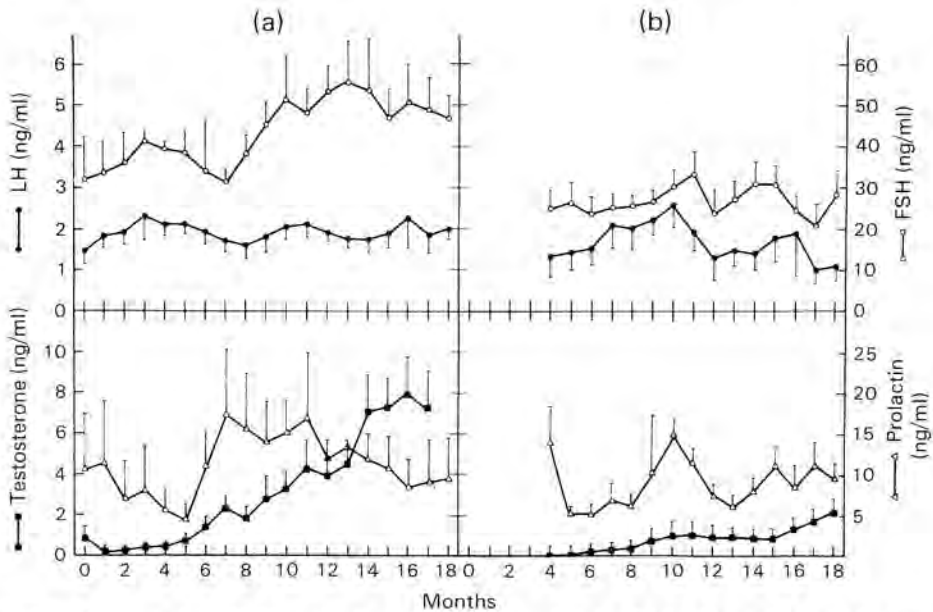
Text-fig. 3. Plasma LH, FSH, prolactin and testosterone levels of (a) 4 Brown Swiss bulls kept in Munich, West Germany and (b) 4 British Friesian bulls kept at Nairobi, Kenya. All points represent the mean ±s.e.m. of 52 samples, measured 3 times weekly except at birth when one sample/animal was taken.

The results from bulls sampled in Nairobi are shown in Text-fig. 3(b). Concentrations of LH and FSH were highest at 10 or 11 and again at 14–16 months. Testosterone levels began to increase at 6 months, but never approached the levels observed in the Brown Swiss breed. Prolactin concentrations decreased sharply between 4 and 5 months of age and were elevated again at 9–10 and at 15–18 months.

*Frequency of pulsatile gonadotrophin secretion (Exp. 3)*

A pulsatile pattern of LH release was obvious during all age periods studied. The longest interval between two pulses occurred during the first month of age (321 ± 49 min). The frequency increased thereafter (2nd month: 219 ± 13 min pulse interval; 5th month: 246 ± 48 min; 10th month: 223 ± 34 min). Higher frequencies coincided with lower amplitudes. There were no differences between male and female animals. About the same number of FSH pulses was noticeable. A small testosterone release followed the gonadotrophin pulses at 1–2 months of age; these were more pronounced at 5–10 months of age.

Pituitary responsiveness to GnRH (Exp. 4)

An LH and FSH response was observed after every injection of 1 μg GnRH/kg. The results are summarized in Text-fig. 4. The LH response increased throughout the experimental period except during Months 6 and 7 whilst the FSH response increased only up to 5 months, before
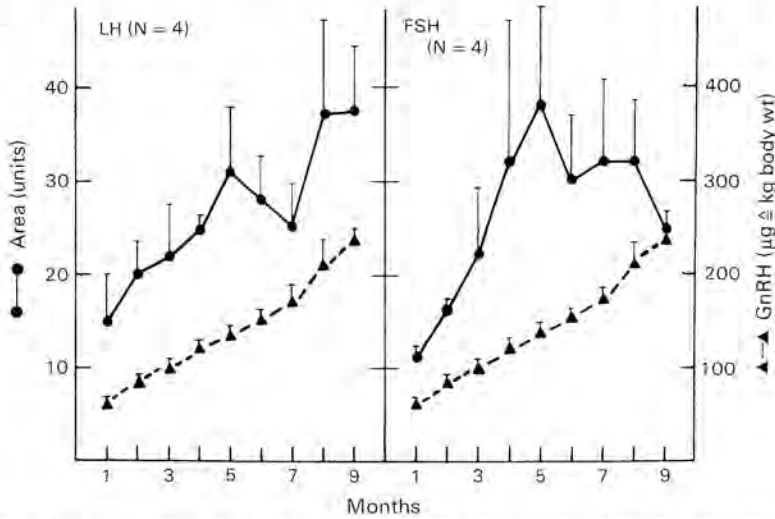
Text-fig. 4. Total gonadotrophin response (measured as area under the dose–response curve) of 2 male and 2 female calves (mean + s.e.m.) given one i.v. injection of 1 μg GnRH/kg body weight once per month.
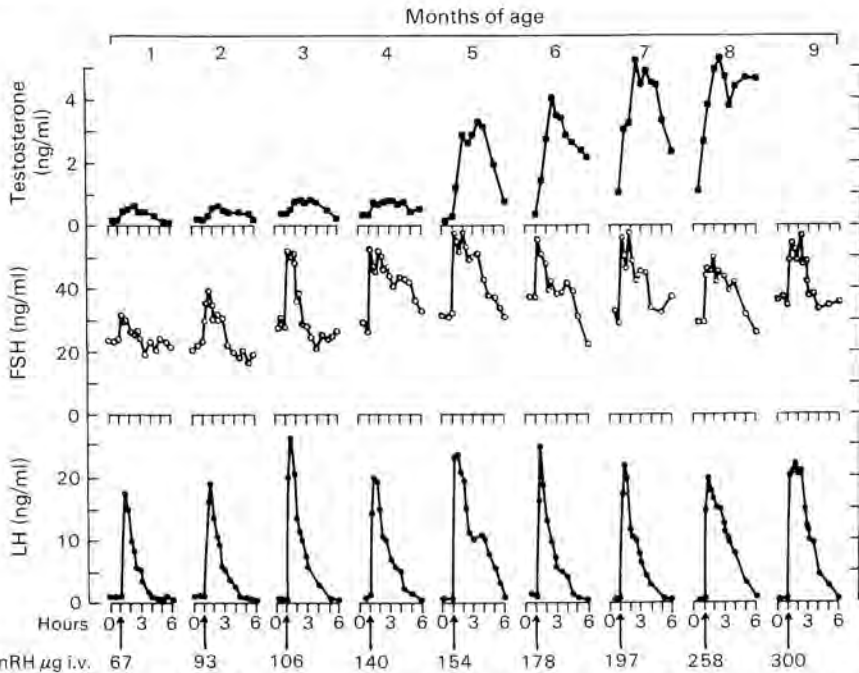
Text-fig. 5. Plasma LH, FSH and testosterone concentrations in a bull given monthly injections of 1 μg GnRH/kg body weight. The arrows indicate the time of the GnRH challenges and the doses involved.

declining. The time from the injection of GnRH to the maximum level of LH increased from 22 $\pm$ 4 min at 1 month to 41 $\pm$ 6 min at 9 months. An example of an individual response pattern to the repeated GnRH injections is presented in Text-fig. 5. A small testosterone response followed the LH release as early as 29 days of age (1 month). The amplitude of the testosterone response remained constant during the first 4 months of life but increased markedly from the 5th month. An increasing gonadotrophin response was also observed after constant injections of 250 µg GnRH, except for a lower FSH release at 10 months of age.

Discussion

Changes in peripheral gonadotrophin and steroid hormone levels take place in both sexes from birth to puberty. After an initial period of increasing LH and FSH values, the pituitary gland secretes reduced amounts of these hormones before puberty. This suppression is gradually overcome and large amounts of gonadotrophins are released when oestrous cycles and sperm production commence (Ortavant, Courot & Hochereau de Reviers, 1977).

Four of the five female animals included in this study exhibited first oestrus during the 10th and 11th month, and this coincides with puberty in the males of this breed. Changes in ovarian response to gonadotrophins do not seem to be of major importance in the timing of puberty. Follicular growth in the gonads starts soon after birth (Desjardins & Hafs, 1968). The ovaries of calves are already able to respond to gonadotrophins as early as 2 months of age (Onuma, Hahn & Foote, 1970). These follicles are able to produce considerable amounts of oestrogens (Karg, Hoffmann, Vogt & Behr, 1972). The low progesterone levels in peripheral blood before 9 months of age seem to be of adrenal origin. Progesterone increases before first oestrus and is probably caused by small releases of LH and FSH. This pattern seems to be common to the resumption of oestrous cycles after periods of anovulation, for example, in beef heifers at puberty (Gonzalez-Padilla, Wiltbank & Niswender, 1975), cows after parturition (Lamming, 1978; Schams *et al.*, 1978b) and sheep (Thorburn, Bassett & Smith, 1969; Walton, Cunningham, Temple & Bowman, 1974) or roe deer (Schams, Barth & Karg, 1980) after seasonal anoestrus. This progesterone rise probably plays a key role in the establishment of a pulsatile pattern of gonadotrophin secretion appropriate for the development of an ovulatory surge.

The pituitary gland is able to release LH and FSH after GnRH treatment at any age even when basal gonadotrophin secretion is low. The period of reduced responsiveness to GnRH (Text-fig. 4) indicates a shift in LH and FSH synthesis and release ratio. Changes in peripheral LH and FSH levels can be induced by changing the frequency of pulsatile GnRH infusion in the rhesus monkey bearing hypothalamic lesions: lower frequencies of infusion favour FSH secretion (Knobil, 1980). The major components of the control systems which govern the development of ovarian function during puberty in cattle seem to act at the level of the hypothalamus and the pituitary gland. It is reasonable to speculate that changes in the inhibitory and stimulatory feedback action of oestradiol on tonic gonadotrophin secretion are involved, as recently demonstrated for the lamb by Foster & Ryan (1979).

A functional hypothalamo–pituitary–gonadal axis in male calves exists as early as Day 28 of life since castration at this age causes a steep rise in LH levels (Bass, Peterson, Payne & Jarnet, 1977). This finding coincides well with our observation that the testes are able to respond with a small testosterone increase to a GnRH injection as early as Day 29 (Text-fig. 5). Also, endogenous LH pulses cause release of testosterone (Karg *et al.*, 1976; Schams, Gombe, Schallenberger, Reinhardt & Claus, 1978a). Our results showing an increase of basal LH concentrations at 3–4 months of age agree with other reports (Lacroix, Garnier & Pelletier, 1977; Lacroix & Pelletier, 1979; McCarthy, Convey & Hafs, 1979a) and are similar to that in rams at about 6 weeks of age (Lee *et al.*, 1976; Foster *et al.*, 1978; Wilson & Lapwood, 1979). McCarthy *et al.* (1979a) and McCarthy, Hafs & Convey (1979b) did not find significant

changes in FSH levels in prepubertal bulls whereas Lee *et al.* (1976) observed two FSH rises in rams. These enhanced FSH concentrations could stimulate spermatogenesis by an interaction through the Sertoli cell (Fritz, 1978; Dorrington & Armstrong, 1978) or may sensitize the testis to respond to LH with testosterone production (Swerdloff & Odell, 1977). Our results for testosterone agree with those of Rawlings, Hafs & Swanson (1972).

Wuttke, Döhler & Gelato (1976) have presented evidence for a functional role of prolactin in the onset of puberty in the rat. A strong seasonal influence on prolactin in cattle could be derived from the data of the 4 male and 5 female Brown Swiss cattle included in the present study (Schams & Reinhardt, 1974) but an increase in prolactin coinciding with the testosterone rise in the males results when the data are grouped according to age (see Text-fig. 3) and not to season, which agrees with the data obtained by McCarthy *et al.* (1979a). A sharp peak of prolactin was observed by Ravault & Courot (1975) in rams at about 80 days of age during a season when low prolactin concentrations would normally have been expected. Whether those changes in prolactin secretion have a functional role in the processes initiating or controlling sexual maturation in cattle cannot be derived from our experiment.

We could not observe major differences in the gonadotrophin secretion pattern stimulating gonadal function during the developmental period between male and female cattle. This leads to the conclusion that the neuroendocrine mechanisms which control onset of gonadal functions are probably identical in both sexes.

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