Plasma oestrogen, progesterone and other reproductive responses of gilts to photoperiods

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Summary. Yorkshire gilts in 18 h cool-white fluorescent light with 6 h dark daily and those in 9.0–10.8 h natural light exhibited puberty earlier (165 and 175 days: $P < 0.05$) and had more corpora lutea (13.5 and 12.6: $P < 0.05$) than those reared in complete darkness (200 days and 11.3 respectively).

Weekly samples of plasma showed significant fluctuations of progesterone which confirmed the different times of the first overt oestrus (puberty). In all 3 groups total oestrogen concentrations showed a peak at about 135 days. The correlation between oestrogen and progesterone values changed from a positive to a negative value at about 135 days of age. It is suggested that the oestrogen peak marks a time of change in sensitivity of the reproductive system to hormonal feedback.

Introduction

Much of what organisms do is temporally organized with respect to the environmental day–night cycle (Menaker, Takahashi & Eskin, 1978). Reports on the effects of photoperiod on reproduction in gilts and sows have been conflicting. While some workers (Surmuhi, Ceremnyh, Timofeev & Poznikova, 1970; Hacker, King & Bearss, 1973) advocate long photoperiods to accelerate puberty and improve conception rates, others (Dufour & Bernard, 1968; Benkov, 1974) advocate short photoperiods to produce the same beneficial effects. Reports on the effect of photoperiod on gonadotrophin and sex steroid release, uterine and gonadal development and cyclic function, and the role of the pineal gland in the mediation of photoperiodic effects on reproductive hormones are available for many species, but studies on the effect of photoperiod on reproduction in pigs have not hitherto included measurement of gonadotrophic and sex steroid hormone concentrations.

Gilts were used in the present study to investigate the effects of various photoperiods on puberty, ovulation rate, and oestrogen and progesterone concentration in blood plasma. LH concentrations in plasma were also measured, but the results are to be reported elsewhere.

Materials and Methods

Animals and management

Yorkshire gilts were assigned to each of 3 photoperiods (6 pigs/group). The photoperiods were complete darkness (CD), 18 h of cool-white fluorescent light (950 lux at 45 cm above floor

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surface) followed by 6 h darkness (LD) and natural photoperiod of 9.0–10.8 h daylight per day (NLD). In Group CD dim red light was present for 1.5 h/day to facilitate experimental routines. The gilts had free access to water from drinking nipples, and feed was restricted only after the gilts had reached 100 kg body weight. All gilts were bled from the suborbital sinus on Day 1 (week 0) of the experiment and at weekly intervals thereafter. Plasma was separated and stored at -20°C until assayed for oestrogens and progesterone.

Gilts were checked for oestrus daily by the elicitation of lordosis by back pressure after gentle rubbing of the flanks. Visual observations for mounting, being mounted, reddening of the vulva and vulval mucus discharge, palpation and fingering of the vulva and, sometimes, the response to presence of a boar at the fence without opportunity for full body contact, were used to supplement and augment the results of the lordosis test. The boars were housed away from the gilts and were brought into the presence of gilts only for purposes of detection of oestrus. The gilts detected in oestrus were artificially inseminated on 2 consecutive days with extended, fresh boar semen. The gilts were slaughtered at approximately 30 days after insemination and the number of corpora lutea in the ovaries was recorded. A final blood sample was collected at the time of slaughter.

Oestrogen and progesterone assays

Total oestrogen and progesterone concentrations in plasma were measured by the radioimmunoassay procedures described by Louis, Hafs & Seguin (1973) and Britt, Kittok & Harrison (1974), with the following modifications. The plasma (200 or 400 μl) was extracted with 4 ml benzene–hexane mixture (2:1, v/v, for oestrogens and 3:1, v/v, for progesterone). All estimations were in duplicate. The oestrogen-specific and progesterone-specific antisera were used at dilutions of 1:60 000 and 1:10 000, respectively, and at these dilutions the antisera bound 50% of their target ^3H -labelled steroids. The cross-reactivities of the specific antisera have been previously reported (Furr, 1973; Erb, Monk, Mollett, Malven & Callahan, 1976). When blank values represented more than 10% of the usable portion of the standard curve, the assay was arbitrarily decided to be invalid (Abraham, 1974). The mean extraction recoveries were 98% for oestrogens and 92% for progesterone. The inter- and intra-assay coefficients of variation were 16.8 and 10.1% respectively for the oestrogen assay and 12.3 and 14.0% for the progesterone assay. The sensitivities were 6 pg oestrogen and 0.025 ng progesterone. The displacement curves for oestrogens and progesterone in various volumes of plasma were parallel to their corresponding standard curves. When known amounts of oestradiol-17 β (10, 20, 40, 60 and 80 pg) and progesterone (0.1, 0.25, 1.0, 1.5, and 2.0 ng) were added to plasma, quantitative recoveries were good; the regression coefficients were 99.64% and 99.17% respectively ($n = 16$).

Statistical analysis

The data were analysed on the original split-plot design, with a time factor included whenever samples were collected over extended time periods. Differences between means were tested by the critical difference method (Steel & Torrie, 1960). The observations on the number of corpora lutea were transformed into square roots (Steel & Torrie, 1960) before being analysed.

Results

The gilts in the 3 groups were of similar age and weight at the start of the experiment (Table 1). Progesterone profiles of 17 of the 18 experimental gilts confirmed the efficiency of the procedure for detection of oestrus. The gilts in Groups LD and NLD attained puberty earlier ($P < 0.05$) than those in Group CD (Table 1). Although the gilts in Group LD tended to reach puberty

Table 1. Body weights, ages and CL number for gilts exposed to different light treatments (see text) from 100 days of age

Group	No. of gilts	Initial		Puberty		Slaughter
		Wt (kg)	Age (days)	Wt (kg)	Age (days)	No. of CL*
CD	6	38.2	103.8	100.7	200.5 ^a	11.3 ^d
LD	6	37.2	103.7	83.3	164.8 ^b	13.5 ^c
NLD	6	36.5	103.6	90.7	175.3 ^b	12.6 ^{cd}
Error M.S. (d.f. 3)		105.944	49.222	262.0	119.950	0.0142†

Means in the same column having different superscripts are significantly different ($P < 0.05$).

* Back-transformed from analysed $\sqrt{x}$ figures.

† On $\sqrt{x}$ transformed data.

earlier than those in Group NLD the difference was not significant. None of the differences in weight was significant. The gilts in Group LD has more ($P < 0.05$) corpora lutea at slaughter than did those in Group CD, but the numbers for Groups CD and NLD did not differ. As shown in Text-fig. 1, the plasma progesterone concentrations fluctuated ($P < 0.01$) during the experiment. Increases from the early low levels began in Weeks 6, 8 and 10 for Groups LD, NLD and CD respectively. The time at which the gilts in each group showed higher plasma progesterone concentrations corresponded to the order (i.e. age) at which they attained puberty.

Text-fig. 1. Mean $\pm$ s.e.m. plasma progesterone concentrations in gilts exposed to constant darkness (Group CD, x), 18 h light/6 h dark (Group LD, ●) or natural day/night (Group NLD, ▲) from 100 days of age (Week 0).

Text-fig. 2. Mean weekly concentrations of total oestrogens in plasma of gilts exposed to constant darkness (Group CD, x), 18 h light/6 h dark (Group LD, ●) or natural day/night (Group NLD, ▲) from 100 days of age (Week 0). The s.e.m. was 1.5–6.7, 1.9–8.0 and 1.0–5.9 pg/ml for values in Groups CD, LD and NLD respectively. The error MS was 393.5 for 51 d.f.

The total oestrogen concentrations in all 3 groups also exhibited fluctuations ($P < 0.01$) of similar pattern (Text-fig. 2). Basal concentrations of about 20 pg/ml increased sharply in Week 5. Concentrations of 141 and 211 pg/ml for 2 gilts in Week 5 caused the very high value in Group NLD. Oestrogen concentrations gradually declined after Week 5 and were low between Weeks 10 and 15. An increase was seen in the last week of the experiment.

The linear relationship of oestrogen (in pg) to progesterone (in ng) during the experiment was also studied. Over the entire experiment (Weeks 1–18, $n = 36$) the linear correlation coefficient was positive and insignificant ($r = 0.1058$, $P > 0.05$). The same was true when only the data of the first 4 weeks were considered ($r = 0.1355$, $P > 0.05$, $n = 72$). From Weeks 5 to 15, the correlation coefficient was negative but still remained insignificant ($r = -0.0753$, $P > 0.05$, $n = 196$).

Discussion

The gilts in Groups LD and NLD were significantly younger at puberty and had higher ovulation rates than those in Group CD. These results confirm earlier reports that photoperiod accelerates puberty in gilts (Hacker *et al.*, 1973; Hacker & Ntunde, 1976) and that in gilts reared in long photoperiods the uteri and ovaries were heavier and the follicular volumes greater than in gilts reared in short photoperiods (Surmuhiin & Ceremnyah, 1970; Surmuhiin *et al.*, 1970).

The maintenance of high levels of progesterone seen in the gilts confirmed that the gilts were pregnant. The delay in the initial elevation of progesterone concentration in the gilts in Groups

NLD and CD, although oestrogen concentrations had increased in all groups in Week 5, seems to reflect delay of ovulation and/or corpus luteum formation and function. The absence of any difference in the LH concentrations and profiles in the gilts reared in the 3 groups (unpublished data) even though the blood sampling frequency was inadequate to detect any LH surges, suggests that a delay in corpus luteum formation and function was the most likely reason for the difference. There is a dearth of published research regarding the circulating concentrations of progesterone and oestrogen in the young gilt. The source of the Week 5 oestrogen surge is likely to be vesicular follicles that are present in the gonads at this time (Casida, 1976). However, other organs capable of metabolizing sex steroids and synthesizing oestradiol, such as adrenals (Bridges & Goldman, 1975) and the pineal gland (Cardinali, Nagle, Gomez & Rosner, 1975; Nagle, Cardinali & Rosner, 1975), may be supplementary sources.

The change of correlation coefficient for total oestrogen and progesterone concentrations from a positive value to a negative value at Week 5 might be an important physiological event. Oestradiol and progesterone levels in sow plasma before and during early pregnancy were linearly and negatively correlated (Guthrie, Henricks & Handlin, 1972), as in the present study. The change at Week 5 probably marks the transition of the gilt from "impuberal" to "prepuberal" (Courot, de Reviers & Pelletier, 1975) gonadal growth and the "turning on" of sensitivity of the hypothalamo-hypophyseal-gonadal axis to hormonal feedback mechanisms.

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