Effect of maternal treatment with altrenogest on pituitary response to exogenous GnRH in pubertal stallions

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Summary. The pituitary response to exogenous GnRH was studied in 8 colts of Quarter Horse phenotype from 32 to 96 weeks of age. Colts were from dams treated daily from Day 20 to 325 of gestation with (1) 2 ml neobee oil per 50 kg body weight (controls); or (2) 2 ml altrenogest per 50 kg body weight. GnRH challenges (5 µg/kg body weight) were administered every 8 weeks from 32 to 96 weeks of age to estimate pituitary content of LH. Blood samples were collected every 20 min for 4 h before GnRH and 15, 30, 45, 60, 90, 120, 180, 240 and 360 min after GnRH. Serum concentrations of LH and FSH were determined for the 2 pre-GnRH and all post-GnRH samples. Baseline concentrations (mean of 2 pre-GnRH samples) of LH and FSH were not affected by treatment ($P > 0.05$). Serum concentrations of LH declined from 40 to 56 weeks and rose again between 72 and 80 weeks. Basal concentrations of FSH declined from 32 to 56 weeks, and varied widely after 56 weeks. The maximum LH response to GnRH (highest concentration after GnRH minus baseline) declined steadily in both groups for 48 to 64 weeks but remained relatively constant in both groups after 64 weeks. The maximum FSH response to GnRH declined from 32 to 64 weeks then remained relatively constant in both groups. The GnRH-induced gonadotrophin release remained low with a transient increase at 72 weeks for both hormones. There was no treatment effect ($P > 0.05$) on time to maximum gonadotrophin release after GnRH, and the time to maximum release varied widely throughout the study. Changes in basal concentrations of LH were associated with changes in LH release after GnRH; whereas basal concentrations of FSH were associated with changes in FSH release only from 32 to 56 weeks of age. The prepubertal changes in secretion of LH in stallions may therefore be associated with a reduction and restoration of the ability of the pituitary to secrete LH as assessed by discharge following exogenous GnRH. However, this relationship does not hold true for FSH after 56 weeks of age.

Keywords: puberty; stallion; exogenous GnRH; LH; FSH

Introduction

Little is known regarding changes in the hypothalamic–pituitary axis during the prepubertal period of the horse. Wesson & Ginther (1980) studied plasma gonadotrophin concentrations in colts from birth to 8 months; however, it is unlikely that hormonal changes prerequisite for puberty would have been detected. Thompson & Honey (1984) measured changes in concentrations of LH, FSH and testosterone from 6 to 26 months of age in Quarter Horse colts, but since age at puberty was not determined, the temporal relationship between hormonal changes and the attainment of puberty could not be ascertained.

The amount of LH released in response to exogenous GnRH is highly correlated with pituitary content of LH in mares (Silvia *et al.*, 1987), and to seasonal hormonal changes in stallions (Clay *et al.*, 1989). GnRH challenges were therefore used to estimate indirectly changes in pituitary

content of LH and FSH during the prepubertal period in colts. The objectives of this study were to: (1) measure changes in basal concentrations of LH and FSH from 32 to 96 weeks; (2) measure changes in secretion of LH and FSH after exogenous GnRH treatment from 32 to 96 weeks; and (3) determine whether progestagen treatment during pregnancy affected the hypothalamic-pituitary axis of the offspring.

Materials and Methods

General. Eight cross-bred, light horse colts born between 20 July and 29 August 1985 were used to study the pituitary response to exogenous GnRH in prepubertal and pubertal stallions. The colts were part of a study to determine effects on offspring of administering an oral progestagen, altrenogest (Regu-Mate: Hoechst-Roussel Agri-Vet Company, Somerville, NJ, USA), to pregnant mares. Pregnant mares were assigned to 1 of 2 groups: (1) controls, receiving 2 ml neobee oil per 50 kg body weight; and (2) 2 ml (2.2 mg/ml) altrenogest per 50 kg body weight. Treatments were administered daily from Day 20 to 325 of gestation. The dosage of altrenogest administered was twice the amount recommended for suppression of oestrus. Four colts were randomly chosen from each treatment group to study the pituitary response to exogenous GnRH. General care of these colts has been described previously (Naden *et al.*, 1990).

GnRH challenges. GnRH challenges were conducted every 8 weeks from 32 to 96 weeks of age. An indwelling catheter was positioned in 1 jugular vein and sutured into place on the day before blood collection. Blood samples were collected at 20-min intervals from 08:00 to 12:00 h before intravenous GnRH administration (LH-RH L-7134; Sigma Chemical Co., St Louis, MO, USA; 5 µg/kg body weight in 0.15 M-NaCl). Blood samples were taken 15, 30, 45, 60, 90, 120, 180, 240 and 360 min after GnRH administration. Samples were allowed to clot at ambient temperature for 1 h, then stored overnight at 5°C. Serum was harvested by centrifugation the following day. Serum samples were stored at -20°C until assayed.

Hormone assays. All pre-GnRH samples were assayed for LH (Nett *et al.*, 1975) and FSH (Nett *et al.*, 1979) for the GnRH challenges administered at 40 and 82 weeks of age. No pulsatile release of LH or FSH was detected, presumably due to infrequent sampling. Therefore, only the 2 samples taken before GnRH administration, and all post-GnRH samples were assayed for LH and FSH for the remaining challenges. The inter-assay coefficients of variation for these assays were 12.4 and 19.0% for LH and FSH, respectively. The lower limit of sensitivity (1 standard deviation below the specific counts bound in buffer control tubes) was 1.50 ng LH/ml and 5.35 ng FSH/ml. A value equal to the sensitivity of the assay was assumed for any sample in which the concentration of hormone was less than the limit of sensitivity. The FSH standard was E276B (donated by H. A. Papkoff) and LH standard was LER-958-1 (donated by L. E. Reichert).

Statistical analysis. Three dependent variables were analysed for each of the 9 different GnRH challenges. First, baseline hormonal concentrations were calculated by averaging the 2 pre-GnRH samples. Second, the maximum LH and FSH response was calculated as the highest concentration of hormone achieved after GnRH, minus the baseline concentration. Third, the time to maximum hormonal response was calculated as the number of minutes from GnRH administration to the highest concentration of gonadotrophin achieved. Differences between treatment groups were determined for each variable within a GnRH challenge by the Wilcoxon rank-sum test (SAS, 1987). Age-related changes were characterized by graphing the median values of each variable across time.

Results

Basal serum concentrations of LH and FSH (Figs 1 & 2) were not different ($P > 0.05$) between treatments. Serum concentrations of LH declined from Weeks 40 to 56 and remained low until 80 weeks (Fig. 1). Basal serum concentrations of FSH also decreased from Weeks 32 to 56, and then were variable after 56 weeks, with an upward trend after 72 weeks of age (Fig. 2).

The maximum LH release after GnRH was lower in the colts from treated mares (treated colts) ($P < 0.05$) at 40 weeks of age (Fig. 1). The maximum LH response to GnRH declined steadily in both treatment groups from 48 to 64 weeks of age (Fig. 1) but remained relatively constant in both groups after 64 weeks.

The maximum FSH response to GnRH administration tended to be lower in treated colts, but the only difference was at 32 weeks of age ($P < 0.05$; Fig. 2). The FSH response declined from 32 to 64 weeks of age, then remained relatively constant in both groups (Fig. 2).

Fig. 1. Basal concentrations of LH (a) and maximum LH response to injection of 5 µg GnRH/kg body weight (b) in treated (▲) and control (●) colts from 32 to 96 weeks of age. Arrow designates treatment differences ($P < 0.05$). The mean standard deviations in control and treated colts were respectively 1.68 and 0.89 for basal concentrations of LH and 12.33 and 3.80 for maximum LH response after GnRH.

The initial decline in basal concentrations of LH preceded the decline in GnRH-stimulated LH release by about 8 weeks (Fig. 1). Conversely, the maximum LH response rose 8 weeks before an increase in basal concentrations of LH. Changes in basal concentrations of FSH preceded changes in GnRH stimulated FSH secretion by 8 weeks; however, after 80 weeks of age, maximum FSH response remained constant while basal concentrations of FSH fluctuated widely (Fig. 2).

There was no effect of treatment ($P > 0.05$) on time to maximum concentrations of LH and FSH after GnRH administration (Table 1). Also, the median time to maximum hormonal concentrations was variable. The time to maximum concentration of LH increased from 20 to 30 min between 32 and 56 weeks of age and fluctuated around 30 min from 56 to 96 weeks of age. The time to maximum concentration of FSH after GnRH administration varied between 30 and 45 min throughout the duration of the study.

Discussion

In the present study, baseline concentrations of LH were not influenced by maternal treatment with altrenogest. The change in serum concentrations of LH is in agreement with longitudinal profiles previously described for these stallions (Naden *et al.*, 1990). The pattern of basal concentrations

Fig. 2. Basal concentrations of FSH (a) and maximum FSH response to injection of 5 µg GnRH/kg body weight (b) in treated (▲) and control (●) colts. Arrow designates treatment differences ($P < 0.05$). The mean standard deviations in control and treated colts respectively were 5.02 and 4.44 for basal FSH concentrations and 24.60 and 11.66 for maximum FSH response after GnRH.

of LH in these stallions (Fig. 1) was similar to those of prepubertal colts (Thompson & Honey, 1984), ram lambs (Olster & Foster, 1986), bulls (Amann & Walker, 1983) and boars (Floracruz & Lapwood, 1978).

The first rise in serum concentrations of LH is a result of increased LH pulse frequency and amplitude in prepubertal bulls (Amann & Walker, 1983) and rams (Olster & Foster, 1986). However, in adult stallions, the seasonal rise in serum concentrations of LH was only due to an increase in LH pulse amplitude (Clay *et al.*, 1988). Further research is therefore necessary to determine changes in the frequency and amplitude of pulsatile LH secretion in prepubertal stallions. This rise in LH is postulated to sensitize Leydig cells to LH and, thus, elevate intratesticular levels of testosterone (Amann & Walker, 1983). These stallions had no significant rise in serum concentrations of testosterone that corresponded to the first increase in concentrations of LH (Naden *et al.*, 1990), suggesting that the Leydig cells are unable to secrete testosterone at this time. Clay *et al.* (1988) reported that seasonal fluctuations in serum concentrations of LH, FSH and testosterone in stallions resulted in low levels of hormones from September to January. The winter months for these colts were from 52 to 72 weeks of age. Therefore, seasonality may have inhibited a rise in testosterone at this time as well as influenced the prolonged depression of serum concentrations of LH from 56 to 80 weeks of age.

Table 1. Median time (min) to maximum LH and FSH response to injection of 5 µg GnRH/kg bodyweight

Week	Time to maximum LH*		Time to maximum FSH†	
	Control	Treated	Control	Treated
32	22.5	15.0	30.0	30.0
40	30.0	30.0	37.5	37.5
48	30.0	15.0	30.0	30.0
56	37.5	30.0	45.0	37.5
64	30.0	30.0	30.0	30.0
72	37.5	22.5	37.5	37.5
80	30.0	30.0	30.0	22.5
88	37.5	30.0	37.5	30.0
96	37.5	30.0	30.0	37.5

There was no difference between treatment groups ($P > 0.05$) at any age.

*The mean standard deviations were 13.00 and 9.52 in control and treated colts respectively. The mean standard deviations were 10.00 and 10.37 in control and treated colts, respectively.

Baseline concentrations of FSH also were not affected ($P < 0.05$) by maternal treatment with altrenogest. The change in serum concentrations of FSH (Fig. 2) was similar to, but much more variable than, that described previously for these colts (Naden *et al.*, 1990) and was similar to that of spring-born colts (Thompson & Honey, 1984) and rams (Olster & Foster, 1986). The increased variability of basal concentrations of FSH in this study, over the companion study (Naden *et al.*, 1990), could have been due to an 8-week sampling interval and to the fact that only 2 samples, collected 20 min apart, were used to calculate baseline values.

Previously, it has been demonstrated (Silvia *et al.*, 1987) that a dose of 2 µg GnRH/kg body weight stimulated a maximal LH response and that this response, as well as integrated area under the LH response curve, was positively and significantly correlated with pituitary concentrations of LH in adult mares. Assuming that a dose of 5 µg GnRH/kg body weight was capable of stimulating a maximal release of LH in prepubertal stallions, the LH response to GnRH was investigated to determine prepubertal changes in the estimated pituitary content of LH.

The amount of LH released in response to exogenous GnRH was lower ($P < 0.05$) in treated colts at 40 weeks of age (Fig. 1) and the amount of FSH released was lower in treated colts at 32 weeks of age ($P < 0.05$; Fig. 2). There was no effect of treatment ($P > 0.05$) on any other reproductive measure, i.e. basal hormonal concentrations, testicular size, sperm production, sexual behaviour, and age at puberty (Naden *et al.*, 1990). Therefore, if prenatal, maternal treatment with altrenogest did affect pituitary responsiveness to exogenous GnRH early in life, the effect was diminished after 40 weeks of age and had no adverse effect on functional reproductive characteristics.

The amount of LH released after GnRH administration increased from 32 to 48 weeks of age (Fig. 1) and was in agreement with reports of an increasing LH response after birth in bulls (Lacroix & Pelletier, 1979) and rams (Galloway & Pelletier, 1974). However, comparisons with other species are difficult because of the disparity of results reported within a species.

In general, a high basal concentration of LH was associated with a high LH response (Fig. 1); however, there was not a total synchrony between the 2 measures. Basal concentrations of LH began to drop before the decline in LH response at 48 weeks and the LH response began to increase before basal concentrations rose at 80 weeks (Fig. 1). The relationship between basal and GnRH-stimulated concentrations of LH was similar to that reported for adult stallions (Clay *et al.*, 1989)

in which baseline concentrations of LH declined, due to seasonal effects, earlier than the maximal LH response to GnRH. A similar phenomenon was reported in mares and was postulated to be due to the decline of GnRH stimulation of the pituitary in the fall being more rapidly revealed as a decline in LH secretion before a marked change in LH synthesis and, therefore, pituitary content (Hart *et al.*, 1984). Conversely, in these colts, it appeared that the synthesis of LH increased before an increase in LH secretion.

GnRH-induced FSH release was lower ($P < 0.05$) in colts from altrenogest-treated dams at 32 weeks of age (Fig. 2). Basal serum concentrations of FSH were closely associated with the maximum FSH response from 32 to 56 weeks of age; but the changes in baseline concentrations of FSH appeared to precede the changes in GnRH induced FSH release by 8 weeks (Fig. 2). After 56 weeks, baseline concentrations of FSH were variable and poorly related to FSH release after GnRH administration. Thus, from 32 to 56 weeks, changes in the hypothalamic-pituitary axis were seen first as a decrease in FSH secretion followed by a decrease in the amount of FSH released by exogenous GnRH treatment. After 64 weeks, the relationship was not clear, since FSH release after GnRH remained low and baseline concentrations of FSH varied widely.

It is questionable whether GnRH-induced FSH release is a good index of pituitary content. Silvia *et al.* (1986) found that pituitary content of FSH in the mare was not affected by season; however, the amount of FSH released after GnRH declined significantly as the breeding season approached (Silvia *et al.*, 1987). Presumably, in the mare, season had no effect on the ability of the pituitary to synthesize FSH, but rather on the release of FSH in response to GnRH. There was no effect of season on GnRH-induced FSH release in adult stallions, whereas an effect of season was demonstrated for baseline concentrations of FSH (Clay *et al.*, 1989). It is therefore difficult to determine whether the changes in FSH released by GnRH in this study were due to changes in pituitary content of FSH or to changes in pituitary sensitivity to GnRH. Since changes in basal concentrations of FSH are closely associated with changes in maximum FSH response from 32 to 56 weeks (Fig. 2), but not after, one might postulate that before 56 weeks changes in serum concentrations of FSH were related to a changing pituitary content of FSH, whereas after 56–64 weeks pituitary sensitivity to GnRH changed and the FSH response to exogenous GnRH was more similar to that of adult stallions.

There was no maternal treatment effect ($P > 0.05$) on time to maximum gonadotrophin response after exogenous GnRH, but the time to maximum gonadotrophin response varied greatly throughout the duration of the study. The time to peak concentrations of LH after exogenous GnRH increased from <30 min to >70 min after 60–80 days of age in ram lambs (Galloway & Pelletier, 1974) and increased after 4 months of age in bulls (Lacroix & Pelletier, 1979). The increased time to maximum LH response occurred at the age when serum concentrations of testosterone first rose in response to increased serum concentrations of LH (Lacroix & Pelletier, 1979). In the present study, time to maximum concentrations of LH tended to increase from 32 to 64 weeks of age, but with great variability. Possibly time to maximum gonadotrophin response did not show a consistent pattern because of the high dosage of GnRH used in this study.

Average age at puberty, as assessed by sperm production, was 83 weeks (Naden *et al.*, 1990). Since spermatogenesis takes 57 days in stallions (Pickett *et al.*, 1989), spermatogenesis was initiated before 73 weeks of age. Both LH and FSH responses to exogenous GnRH had transient increases around 72 weeks of age, then remained low until the end of the study (Figs 1 & 2). The increases in gonadotrophin response to exogenous GnRH were coincident with the rise in serum concentrations of testosterone (Naden *et al.*, 1990). The rise in gonadotrophin response preceded the rise in baseline concentrations of gonadotrophins, which presumably, initiated testosterone production and spermatogenesis at this time. The decrease in the amount of gonadotrophins released in response to exogenous GnRH after 80 weeks of age was probably due to modulation of gonadotrophin synthesis and secretion by testosterone.

The use of GnRH challenges has provided insight regarding changes in the hypothalamic-pituitary axis during the prepubertal period in stallions. However, further research is necessary to

determine how seasonality affects temporal relationships between hormonal changes and the attainment of puberty. Also, the measurement of pituitary content of gonadotrophins, hypothalamic content of GnRH, and pituitary concentrations of GnRH receptors may provide further insight into the regulation of the onset of puberty in the stallion.

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