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Feedback Regulation of Gonadotropic Hormone Secretion in Neonatal Pigs

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ABSTRACT

The effect of castration and of administration of charcoal-treated porcine follicular fluid (pFF) containing inhibin-like activity on plasma concentration of gonadotropic hormones was studied in neonatal pigs. Plasma follicle-stimulating hormone (FSH) concentration averaged 25.1 ± 1.5 ng/ml (mean ± SEM) in 1-wk-old females and gradually declined to 20.2 ± 0.7 ng/ml 6 wk later. Ovariectomy did not significantly influence plasma FSH concentration. In males, concentration averaged 8.0 ± 0.7 ng/ml before castration but rose significantly within 2 days after castration. Injection of luteinizing hormone-releasing hormone (LHRH) did not influence plasma FSH concentrations in intact males, but did in females and in 7-wk-old males castrated at 1 wk. Plasma luteinizing hormone (LH) concentrations in 1-wk-old females (2.2 ± 0.4 ng/ml) gradually declined and were not influenced by castration. Concentrations of plasma LH in 1-wk-old male piglets (2.8 ± 0.7 ng/ml) were not significantly influenced by castration within 2 days but were significantly higher 6 wk later. LHRH induced a significant rise in plasma LH concentrations in all animals.

Injection of pFF resulted in a decline of plasma FSH concentrations in intact and castrated males and in intact females, but did not influence plasma LH concentrations.

These data demonstrate a sex-specific difference in the control of plasma FSH, but not in plasma LH concentration in the neonatal pig. Plasma FSH concentrations, but not plasma LH concentrations, are suppressed by testicular hormones in 1-wk-old piglets. Plasma FSH concentrations can be suppressed in both neonatal male and female pigs by injections of pFF.

INTRODUCTION

The secretion of gonadotropic hormones in the adult animal is partly regulated by gonadal hormones. Androgens, estrogens and progestogens can suppress peripheral concentrations of plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both in males and females, whereas in females estrogens and progestogens can also have a stimulatory effect (for review, see diZerega and Sherins, 1981; Schneider and Leyendecker, 1983). A nonsteroidal gonadal hormone, inhibin, is also involved in the regulation of peripheral concentrations of FSH. Inhibin appears to be a glycoprotein (Jansen et al., 1981). It is present in gonadal fluids (de Jong and Sharpe, 1976; Setchell et al., 1977) and is produced

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by Sertoli cells and granulosa cells in vitro (Steinberger and Steinberger, 1976; Erickson and Hsueh, 1978). Inhibin has a regulatory role in follicular development (Hermans et al., 1980, 1981), while in male rats the FSH suppressive effect of inhibin appears to decrease towards the pubertal period (Hermans et al., 1980).

In the pig, gonadotropic hormone concentrations, both in the plasma and in the pituitary, rise in the late fetal period, when concentrations of steroids in the plasma are high (Colenbrander et al., 1977, 1978, 1982b; Silver et al., 1979; Hennen et al., 1982). The elevated plasma concentration of LH declines shortly after birth, when a functional feedback system appears to be established. Castration of male pigs affects plasma LH concentrations from about 3 wk of age onwards (Colenbrander et al., 1977; Ford and Schanbacher, 1977), while in neonatally ovariectomized gilts LH increases between 80 and 135 days (Wise et al., 1981). Ovarian inhibition of both LH and FSH secretion in gilts was found to be initiated between 8 and 16 wk of age (Wise and Zimmerman, 1982). No obvious sex difference in plasma LH concentrations exists during the perinatal period. However, plasma FSH concentrations in fetal male pigs are significantly lower than in females. Moreover, the increase of FSH concentrations in the plasma following LHRH administration in fetal female, but not in fetal male, pigs underlines this sex difference (Colenbrander et al., 1982a).

These observations suggest a suppression of FSH but not LH secretion in male pigs as early as the fetal and neonatal period; this might be caused by testicular inhibin secretion. However, data on the role of inhibin in regulation of gonadotropin secretion during the fetal and neonatal period are scarce, and mainly concern the rat, in which injection of steroid-free ovarian follicular fluid and of gonadal steroids suppresses peripheral FSH and LH concentrations, respectively (Hermans et al., 1980). The possible involvement of inhibin in the regulation of FSH secretion in neonatal animals can well be studied in the pig, where secretory patterns of FSH and LH diverge in a period when a negative feedback action of gonadal steroids does not seem to exist.

We therefore investigated the effects of castration and administration of charcoal-treated porcine follicular fluid (pFF), which contains inhibin-like activity (Welschen et al., 1977), on plasma concentrations of gonadotropic hormones in neonatal pigs. As the sex-specific difference in FSH concentrations may be more distinct after LHRH treatment, the effect of an intramuscular injection of this releasing hormone on plasma LH and FSH concentrations was also studied in intact and castrated neonatal piglets.

MATERIALS AND METHODS

Animals

In the first experiment, male (n=12) and female (n=12) piglets (crossbred Dutch Landrace × Yorkshire) were castrated or sham-operated at 1 wk of age under halothane/N_2O anesthesia. Blood samples were taken by jugular vein puncture before, and 2, 5, 8, and 24 h after, surgery. Additionally, the effect of LHRH (i.m.) was studied at 2 days (2.5 μg LHRH/kg body weight) and at 42 days (1 μg LHRH/kg body weight) after surgery . Blood samples were taken before, and 30 min after, treatment.

In a second experiment, 5 male and 6 female 1-wk-old piglets were sham-operated (Day 0) and injected with charcoal-treated pFF (3 ml/kg i.p.) at Days 0, 1, and 2; additionally, 5 1-wk-old male piglets were castrated and injected with saline as controls. Blood samples were taken by vein puncture at Day 0, 1, and 2, and weekly thereafter. Following the third injection of pFF at Day 2, blood samples were taken after 5, 8, and 24 h. At 42 days, all animals were injected i.p. with physiological saline (3 ml/kg) and at 43 days with pFF (3 ml/kg). Blood samples were taken before, and 5, 8, and 24 h after, injection.

Follicular Fluid

The porcine follicular fluid was aspirated from ovarian follicles with diameters < 6 mm and treated with charcoal to remove steroids. The concentration of inhibin in this pFF was 0.6 of that in standard bovine FF, as measured in an "in vitro" bio-assay system, using dispersed pituitary cells (de Jong et al., 1979).

Hormone Determinations

Plasma LH and FSH concentrations were measured by radioimmunological methods described previously (van de Wiel et al., 1981).

Statistical Analysis

Sex differences were tested by comparing the means of the male and female groups at the different points in time where observations were collected with a joint significance level of 0.05. The Bonferroni inequality was used to relate joint probabilities in time to marginal probabilities and an inequality by Banerjee (1961) was employed to overcome any difficulties arising from different variances. Castration effects were tested by the approximate degrees of freedom (APDF) method, an approximate extension of Student's t-test to the case of unequal variances (Welch, 1947). Comparisons within groups were made with the paired t-test, comparisons between groups with the APDF method. LHRH effects were tested with the paired t-test. Differences in FSH levels between sexes and between intact or castrated animals were tested by constructing joint 95% confidence intervals for the pairwise differences between the means of the four groups with the Games-Howell method (Dunnett, 1980).

RESULTS

Effects of Castration and/or LHRH

FSH (Fig. 1). At 1 wk of age plasma FSH concentrations in female pigs were 25.1 ± 1.5 ng/ml (mean ± SEM). These high values declined during the first 6 wk of life and averaged 20.2 ± 0.7 ng/ml at 42 days. Castration of females did not significantly increase plasma FSH concentrations, which averaged 22.5 ± 3.2 ng/ml, 22.2 ± 1.8 ng/ml and 21.9 ± 0.7 ng/ml before, 2 days after, and 42 days after surgery, respectively. LHRH induced a significant rise in plasma FSH concentration in females ($p<0.05$) at 2 days and 42 days to 29.6 ± 2.8 ng/ml and to 24.7 ± 0.7 ng/ml, respectively. Castration did not significantly change the response of plasma FSH to LHRH.

In males, plasma FSH concentrations averaged 8.0 ± 0.7 ng/ml before castration and rose significantly to 11.2 ± 1.5 ng/ml at 2 days after surgery and to 24.3 ± 2.3 ng/ml at 42 days of surgery. In controls animals, concentrations averaged 10.5 ± 1.5 ng/ml, 9.9 ± 1.1 ng/ml and 5.6 ± 0.6 ng/ml at 0, 2, and 42 days after sham operation, respectively.

Injection of LHRH did not significantly change plasma FSH in intact males. In castrated males, however, the effect of LHRH was significant at 42 days. At this age, concentrations in castrated males were comparable to those in females and castrated females.

LH (Fig. 2). Plasma LH concentrations at the start of the experiment were 2.8 ± 0.7 ng/ml in males and 2.2 ± 0.4 ng/ml in females.

Castration did not significantly influence concentrations within 2 days (Fig. 2). At 42 days, castrated males had LH levels (3.9 ± 0.7 ng/ml) significantly higher than those in sham-operated controls. In females, such an effect of removal of the gonads was not observed. LHRH induced a significant rise in plasma LH 30 min after injection in all animals, both at 2 days and at 42 days.

Effects of Treatment with Porcine Follicular Fluid

FSH (Fig. 3). Injection of females with charcoal-treated pFF resulted in a gradual decline of plasma FSH concentration from pretreatment levels (16.5 ± 0.8 ng/ml) to 11.9 ± 0.7 ng/ml at 8 h after the last pFF injection ($p<0.05$). A similar treatment also significantly suppressed plasma FSH concentrations

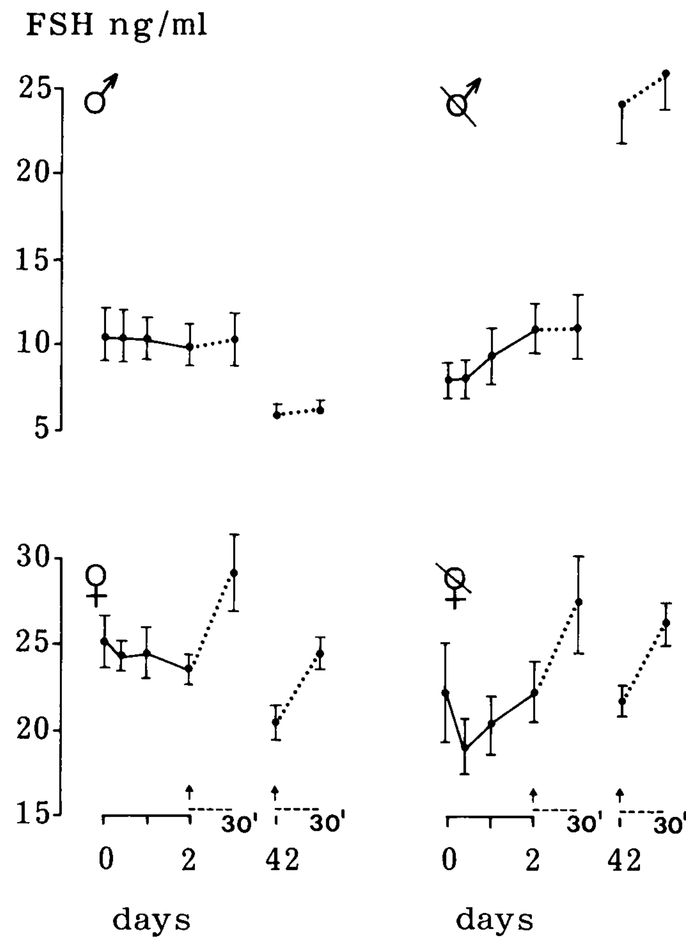

FIG. 1. Changes in plasma follicle-stimulating hormone (FSH) concentrations (mean ± SEM) in male and female pigs after sham operation (♂,♀) or castration (⚦,⚨) at 1 wk of age (Day 0), and the response 30 min after injection of luteinizing hormone-releasing hormone (LHRH, *black arrow*) at Days 2 and 42.

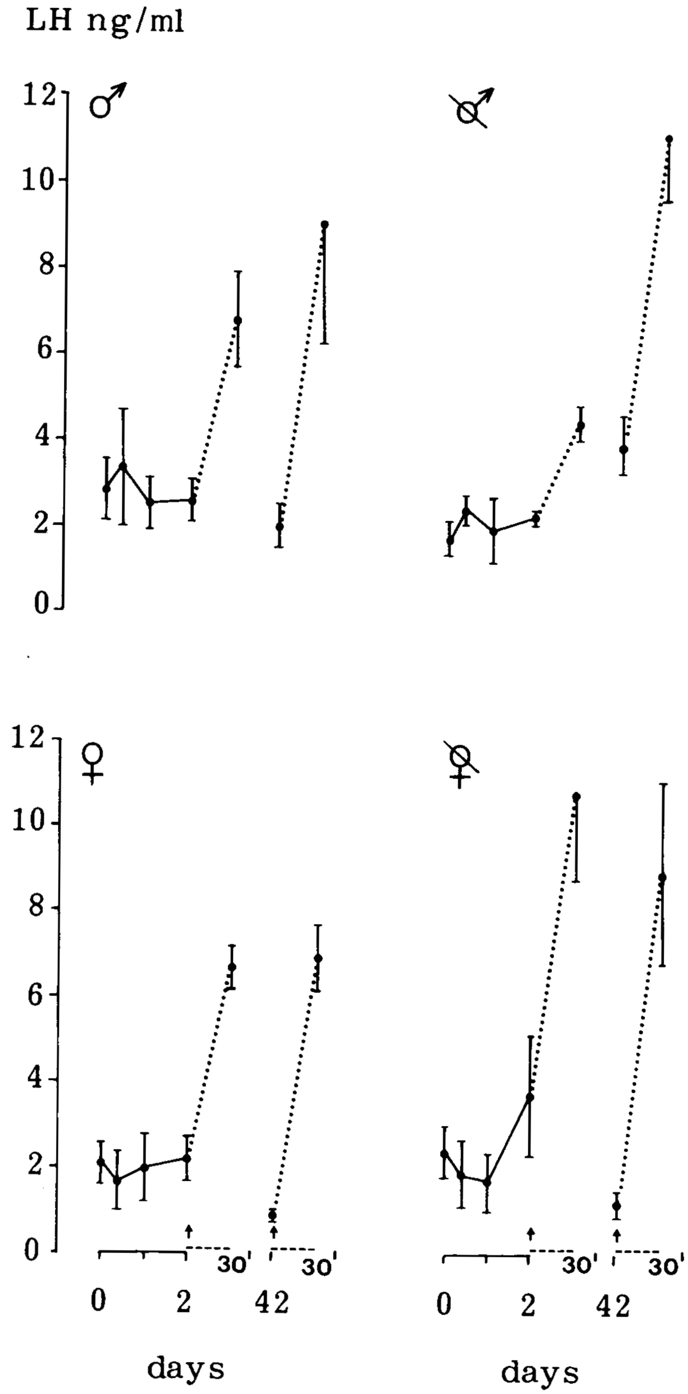

FIG. 2. Changes in plasma luteinizing hormone (LH) concentrations (mean ± SEM) in male and female pigs after sham operation (♂,♀) or castration (♂̸,♀̸) at 1 wk of age (Day 0), and the response 30 min after injection of LH-releasing hormone (LHRH, ***black arrow***) at Days 2 and 42.

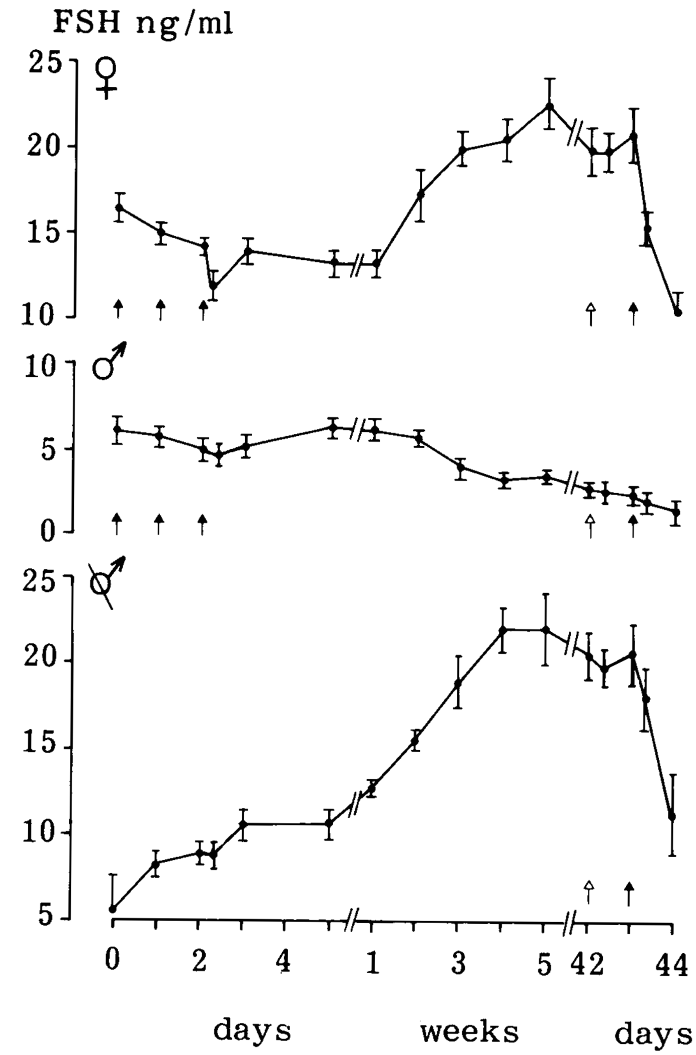

FIG. 3. Changes in plasma follicle-stimulating hormone (FSH) concentrations (mean ± SEM) in pigs after castration (♂̸) or sham operation (♂,♀) at 1 wk of age (Day 0), and the response to porcine follicular fluid (pFF, ***black arrow***) and saline (*white arrow*) injections.

in male pigs from 6.2 ± 0.7 ng/ml to 4.0 ± 0.3 ng/ml 8 h after the last pFF injection.

In male pigs, castration again caused a significant rise of plasma FSH within 3 days when concentrations averaged 10.6 ± 0.7 ng/ml ($p<0.05$). In castrated males, concentrations continued to rise to a level comparable to that in females. Injection of physiological saline did not depress plasma FSH levels within 8 h at 42 days. However, pFF induced a gradual decrease in plasma FSH concentration in intact and castrated males and in intact females within 8 h. The effect was maximal after 24 h, when concentrations for the three groups averaged 1.6 ± 0.5 ng/ml, 11.3 ± 2.1 ng/ml and 10.5 ± 1.2 ng/ml, respectively.

LH (Fig. 4). Plasma LH concentrations were not significantly influenced by treatment with pFF in any of the experimental groups. Plasma LH remained relatively stable in the intact animals. Only in castrated

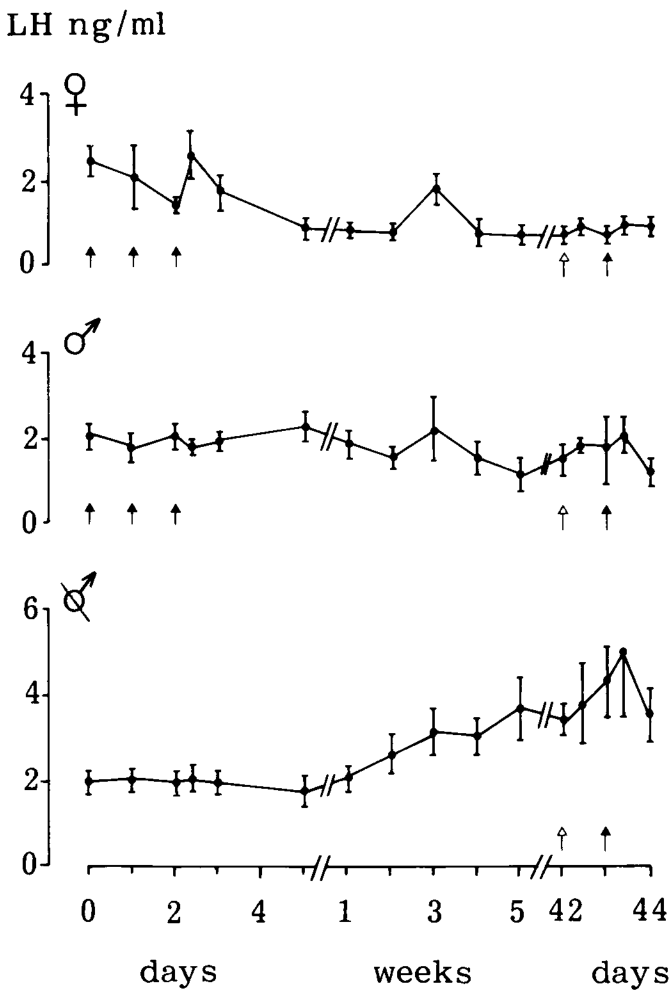

FIG. 4. Changes in plasma luteinizing hormone (LH) concentrations (mean ± SEM) in pigs after castration (⚲) or sham operation (♂,♀) at 1 wk of age (Day 0), and the response to porcine follicular fluid (pFF, *black arrow*) and saline (*white arrow*) injections.

males did LH concentrations gradually rise. The difference with pretreatment levels of plasma LH, although remarkable at 3 wk, became statistically significant at 5 wk postcastration.

DISCUSSION

As a result of perinatal maturational events, LH and FSH concentrations in the plasma of neonatal pigs are high when compared with concentrations of these hormones in the late fetal period (Colenbrander et al., 1978, 1982b; Hennen et al., 1982). The course of FSH levels in the perinatal and neonatal period differs for males and females. Female pigs have high levels of FSH up until 125 days (Camous et al., 1985) or even 24 wk of age (Colenbrander et al., 1982b), while males have high levels of FSH shortly after birth that rapidly decline towards a nadir between 8 and 12 wk. Thereafter, FSH increases slightly towards a relatively constant level, which is maintained until 24 wk of age or older (Colenbrander et al., 1982b). Sex-specific differences in gonadotropic function have been documented also in fetal pigs, where injection of LHRH elicits increased FSH secretion in females but not in males (Colenbrander et al., 1982a). It is likely that these differences in FSH secretion are due to sex differences in the feedback regulation of gonadotropic pituitary function.

Ford and Schanbacher (1977) observed increases of plasma LH only by 3 wk after castration of neonatal boars, whereas Wise et al. (1981) did not find increases of plasma LH until 80 days after castration of neonatal females. These results are consistent with the present observation of increased LH levels by 5 wk after castration of neonatal boars (Fig. 4) and nonaffected LH levels in castrated females during the 42 days of the experiment (Fig. 2). This sex-specific difference of LH response to castration might be explained on the basis of differences in gonadal steroid production. Both histochemical data (van Straaten and Wensing, 1978; Colenbrander et al., 1983) and serum steroid profiles (Colenbrander et al., 1978; Ford, 1983; Camous et al., 1985) point to high steroidogenic activity of neonatal pig testes and low steroidogenic activity of neonatal ovaries. Despite the possibility of inhibition of LH secretion by high doses of exogenous estradiol in newborn (Elsaesser and Parvizi, 1979) and 4- to 20-wk-old female pigs (Wise and Zimmerman, 1982), ovarian steroid feedback on gonadotropic secretion is unlikely to be functioning in neonatal female pigs.

Feedback regulation of FSH secretion involves steroids (Campbell and Schwartz, 1977) and inhibin (de Jong et al., 1978; De Paolo et al., 1979). The decrease of FSH levels during (pre)pubertal development in both intact boars (present study, Figs. 1 and 3; Colenbrander et al., 1982b) and gilts (Camous et al., 1985) might point to the development of gonadal feedback regulation of FSH secretion. The absence of an increase of plasma FSH by 42 days after castration of females in the present study indicates that the negative feedback regulation of FSH secretion is not functioning in neonatal females, and that ovarian inhibin has no apparent role in regulation of FSH at this age. This is consistent with morphological data on ovarian development, since antral follicles with granulosa cells are present only from 60 days onwards (Oxender et al., 1979). Results of Redmer et al. (1984, 1985, 1986) indicate that at 130 days of age, inhibin may play an important role in the regulation of FSH in prepubertal gilts. The dramatic increase of plasma FSH by 42 days after castration of males, however, indicates that gonadal feedback regulation of FSH secretion is present in neonatal males. The possible involvement of inhibin in this gonadal feedback regulation is indicated by the effect of steroid-free pFF on plasma FSH in castrated males.

Divergent responses of LH and FSH to LHRH are well documented in a number of species (de Kretser et al., 1983) and have been reported also in the fetal pig (Colenbrander et al., 1982a). FSH responses to LHRH in neonatal male pigs seem to be inhibited by testicular hormones, since the increase of plasma FSH that follows LHRH treatment of intact females and castrated males at 42 days is absent in intact males.

Responsiveness of neonatal FSH secretion to exogenous inhibin-like activity is apparent in both male and female pigs. Increasing sensitivity to pFF after previous exposure to pFF, as observed in immature female rats (de Jong et al., 1978), may also occur in the pig. A steady decline of FSH levels occurred during the three days of pFF treatment, and in 1-wk-old pigs, lowest FSH levels were found 8 h after the last injection of pFF. Hypersecretion of FSH by 24 h after treatment, as observed in the intact female rat (Hermans et al., 1981), did not occur in the neonatal pig. At 42 days, the lowest FSH values were found 24 h after injection of pFF. This prolonged inhibitory effect of pFF at 42 days might partly be explained by an increased sensitivity with age of the pituitary to pFF, as has been reported for female rats (Hermans et al., 1981).

A well-functioning feedback of inhibin on the pituitary would be of biological significance in adult females, as this mechanism has a role in compensatory ovarian hypertrophy (Redmer et al., 1984, 1985, 1986) and probably regulates the number of follicles that ovulate (van de Wiel et al., 1983).

In adult males inhibin plays a role in regulating FSH secretion. Clinical conditions with increased FSH but normal LH levels have been related to seminiferous tubular dysfunction and defective inhibin feedback on FSH secretion (Setchell et al., 1977; de Kretser et al., 1983). However, the biological significance of inhibin is supposed to be greater in prepubertal males when the blood testis barrier is not yet formed and inhibin may have easy access to the circulation (Waites and Gladwell, 1982) in a period when the Sertoli cell number still increases (Hermans et al., 1980). In this period the feedback of inhibin is slowly diminishing and is involved in determining testis size (Walton et al., 1980).

The biological significance of the regulation of FSH in neonates in unknown. However, our data demonstrate a sex-specific difference in the control of plasma FSH but not of plasma LH concentrations in the neonatal pig. Plasma FSH concentrations increase after removal of the testes while they can be suppressed in both neonatal males and females by charcoal-treated pFF containing inhibin-like activity. This indicates that the neonatal testis is responsible for both the low plasma concentration of FSH and the lack of FSH response to LHRH found in male neonates. It is likely that these effects of the testis are exerted through the secretion of inhibin.

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