

## **ENDOCRINE CONTROL OF SEXUAL MATURATION IN THE FEMALE PIG AND SEXUAL DIFFERENTIATION OF THE STIMULATORY OESTROGEN FEEDBACK MECHANISM**

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Sexual maturation, culminating in puberty and fertility, is a complex process which may involve maturational changes at all levels of the central nervous system-hypothalamo-pituitary-gonadal axis. It is the purpose of this review, based on published and unpublished work from our own laboratory, as well as the work of others, to summarize our present knowledge of the control of sexual maturation in the female pig. Although several other pituitary hormones and adrenal function may be relevant to the process of sexual maturation, because of the restricted availability of such data in the pig only the control of gonadotrophin secretion and ovarian function will be considered. Where information necessary for the understanding of sexual maturation in the pig is lacking, references will be made to work in other species. However, no attempt is made to integrate these data into a general concept of sexual maturation.

The description of the ontogeny of ovarian morphology and steroidogenesis will be followed by a consideration of the developmental pattern of gonadotrophin secretion, attempting to correlate them with the maturation of ovarian function and changes in the responsiveness of the ovary to gonadotrophins. Another aspect to be discussed is the responsiveness of the pituitary to luteinizing hormone (LH) releasing hormone at different stages of development. Special attention will be paid to the ontogeny of negative and positive gonadal steroid feedback control of gonadotrophin secretion as well as to the sexual differentiation of the LH surge mechanism.

### **Development of ovarian function and gonadal steroid levels**

As shown by Allen (1904) the porcine foetal gonad becomes differentiated at about 31–32 days of gestation to an ovary which contains egg nests. In a recent study Oxender *et al.* (1979) have examined ovarian development during the foetal period and postnatally for up to 90 days of age (*Figure 5.1*). The percentage of egg nests observed in the ovary decreased as foetal age increased and egg nests were seldom observed in ovaries from pigs 20 or more days after birth. Starting at about 60–70 days of gestation primordial follicles became the dominant oogonic structure, accounting for

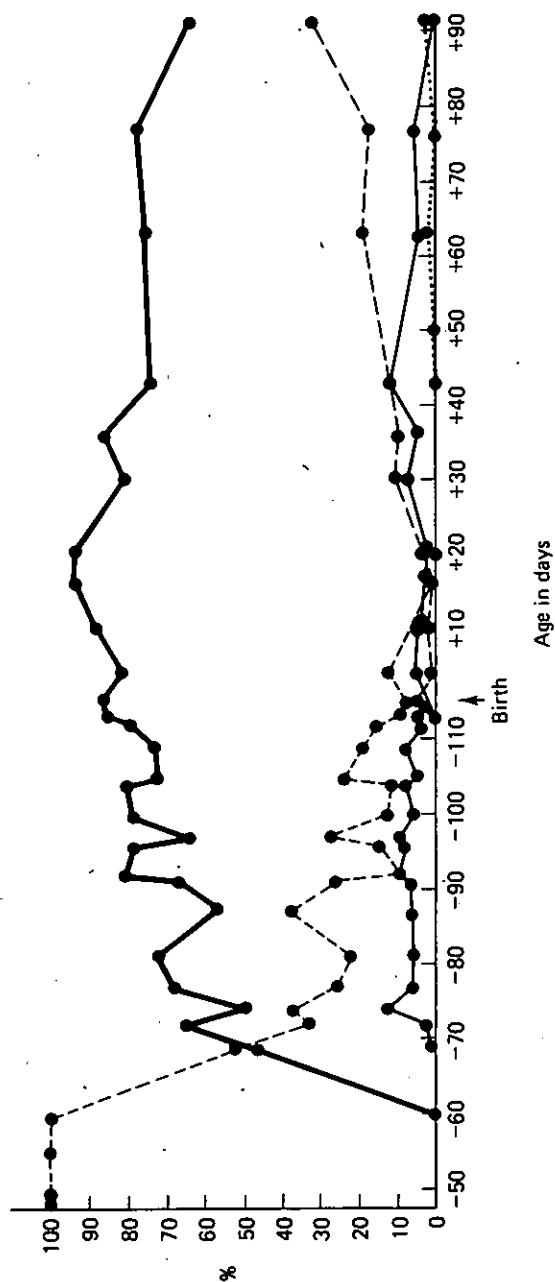


Figure 5.1 The number of oogenic structures in ovarian section from pigs 49 days post coitum to 92 days post partum graphed as a percentage of the total. — Primordial follicles, --- primary follicles, ---- secondary follicles, ..... tertiary follicles. Courtesy of Oxender *et al.* (1979)

about 80% of the ovarian follicles until 90 days after birth. The first primary follicles appeared in ovarian sections from foetuses at 70 days of gestation, whereas secondary follicles were initially observed perinatally and by 90 days postnatally accounted for nearly 30% of the total oogonic structures. Tertiary follicles were never observed in pigs younger than 60 days of age and one or more tertiary follicles were found in only a few of the ovarian sections from pigs of 60–90 days of age.

Although it is not known exactly when ovarian steroidogenic ability develops in the pig, it appears that the ovary matures later than the testis in this respect. Histochemical studies in which the activity of two key enzymes for steroidogenesis were measured (Oxender *et al.*, 1979) revealed that from 49 days of gestation to at least 90 days after birth neither 3 $\beta$ -hydroxysteroid dehydrogenase nor 17 $\beta$ -hydroxysteroid dehydrogenase activity was detectable in the ovaries.

The foetus is no doubt exposed to high levels of oestradiol and progesterone and the levels of both steroids further increase towards the end of gestation. However, available data suggest that the placenta is the major site of production (Barnes, Comline and Silver, 1974; Elsaesser *et al.*, 1976; Macdonald *et al.*, 1979; Macdonald *et al.*, 1980; Choong and Raeside, 1974). The physiological significance of these high sex steroid levels in the foetal circulation remains to be determined and it is unknown whether they exhibit feedback effects on gonadotrophin release.

Within six days of birth, plasma oestradiol declines to undetectable levels ( $\leq 20$  pg/ml) and is still undetectable at 60 and 160 days of age (Elsaesser and Foxcroft, 1978; Elsaesser and Parvizi, 1979). Recent observations (Stickney, 1982) seem to indicate a small increase in concentrations of plasma oestradiol, however, from about 6 pg/ml to 16 pg/ml between 150 and 210 days of age.

Plasma progesterone levels seem to follow a similar pattern to that described for oestradiol-17 $\beta$ . Immediately after birth, levels decline and remain low throughout the prepubertal period (Elsaesser *et al.*, 1976; Elsaesser, Parvizi and Ellendorff, 1978), although occasionally higher levels are observed. Again, information on the immediate prepubertal period is lacking.

At this point it is appropriate to recall that the level of a given hormone is determined by its secretion rate, its interconversion from precursors and its metabolic clearance. In the gilt there is evidence to suggest that the metabolism of oestradiol changes with maturity. Identical doses of oestradiol benzoate/kg body weight produced higher concentrations of oestradiol in the plasma of gilts at 60 days of age than in piglets 6 days old and the highest levels were recorded in gilts at 160 days of age (Elsaesser and Foxcroft, 1978; Elsaesser and Parvizi, 1979). In a recent collaborative study (Elsaesser, Stickney and Foxcroft, 1982) using an isotope infusion technique, the metabolic clearance rate of oestradiol/kg body weight was found to be higher in immature than in peripubertal gilts (Table 5.1).

If the rate of inactivation of oestradiol increases more slowly with age than does body weight and therefore blood volume, constant levels of oestradiol can be achieved without the necessity for the ovary to increase its oestradiol production. Thus, in addition to any direct activation of

**Table 5.1** METABOLIC CLEARANCE RATE (MCR) AND PRODUCTION RATE (PR) OF OESTRADIOL-17 $\beta$  IN GILTS ( $\bar{x} \pm \text{SEM}$ )

Age (days)	Body weight (kg)	n	Infusion material	MCR		Conversion rate $E_1/E_2 \times 100$ (%)	% binding of oestradiol in plasma	PR (ng/min)
				(ml/min)	(ml/min/kg)			
60	19.7 $\pm$ 1.2	5	Oestradiol	1173 $\pm$ 311 <sup>(a)(b)</sup>	62.6 $\pm$ 18.8 <sup>(a)</sup>	33.3 $\pm$ 4.2 <sup>(a)</sup>		37.2 $\pm$ 15.5
			<sup>3</sup> H-Oestradiol	2133 $\pm$ 274	116.0 $\pm$ 14.5 <sup>(a)(b)</sup>	31.6 $\pm$ 3.7 <sup>(b)</sup>	79.8 $\pm$ 1.4	62.5 $\pm$ 15.6 <sup>(a)</sup>
160	66.0 $\pm$ 2.9	4	Oestradiol	2938 $\pm$ 679 <sup>(a)</sup>	47.9 $\pm$ 10.4	14.3 $\pm$ 3.3 <sup>(a)</sup>		108.1 $\pm$ 33.0
			<sup>3</sup> H-Oestradiol	3027 $\pm$ 340 <sup>(b)</sup>	48.5 $\pm$ 4.8 <sup>(b)</sup>	15.9 $\pm$ 2.1 <sup>(b)</sup>	81.3 $\pm$ 1.1	111.5 $\pm$ 7.9 <sup>(a)</sup>

Values with the same superscript are significantly different from each other ( $P$  at least  $\leq 0.05$ )

Courtesy of Elsaesser, Stickney and Foxcroft (1982)

ovarian steroidogenesis, a decrease in the metabolic clearance rate facilitates the elevation of gonadal steroid levels during puberty.

### **Developmental patterns of luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin in the pituitary and plasma and the control of ovarian development**

In view of the well-known stimulatory effects of gonadotrophins on the ovary in the mature animal, the question arises whether there is any direct evidence that the foetal pituitary secretes gonadotrophins and if so, is there any evidence to suggest that follicular development is controlled by the pituitary? Prior to day 80 of gestation gonadotrophin activity in the foetal pituitary is low or undetectable as shown by bioassay (Smith and Dortzbach, 1929; Melampy *et al.*, 1966) and more recently by the histochemical studies of Liwska (1975). Thereafter gonadotrophic activity (Smith and Dortzbach, 1929) or LH concentrations in the anterior pituitary (Melampy *et al.* 1966) increase. Cell types probably equivalent to FSH secreting cells have been described in foetuses at 70–79 days of gestation and were observed sporadically at day 51 of gestation (Liwska, 1978).

This pattern of gonadotrophic activity in the pituitary corresponds well with the changes of plasma LH and FSH concentrations during foetal life (Figure 5.2). Circulating LH concentrations before day 80 of gestation are low or undetectable (Elsaesser *et al.*, 1976; Colenbrander, *et al.*, 1977). During the last few weeks of foetal life LH levels increase, probably a few days earlier in females than in males, and remain elevated for some time after birth (Colenbrander *et al.*, 1977; Elsaesser, Parvizi and Ellendorff, 1978). Somewhat lower LH concentrations are found during the last three weeks of gestation in chronically catheterized pig foetuses (Macdonald *et al.*, 1979).

Serum FSH levels in the foetus behave similarly to the pattern described for LH. Before day 80 of gestation serum FSH concentrations are low. After 80 days a sharp increase in serum FSH concentrations occurs in female foetuses, but a much slower rise occurs in male foetuses. The levels remain relatively constant after birth (Colenbrander, van de Wiel and Wensing, 1980).

To date the pattern of LH and FSH secretion has not been described in detail throughout the whole period from birth to puberty and information from the period immediately before first ovulation is especially lacking. During the first five weeks of life, average LH concentrations decrease in Landrace pigs as well as in miniature pigs. Although miniature pigs mature younger than Landrace pigs, with first oestrus occurring at about 15–20 weeks of age, the mean LH levels appear to remain unchanged during the period between 5 and 24 weeks of age (Colenbrander *et al.*, 1977; Elsaesser *et al.*, 1976; Elsaesser, Parvizi and Ellendorff, 1978).

The developmental pattern of FSH release differs from that of LH. After birth plasma FSH gradually rises to a maximum at about 10 weeks of age and then appears to decline slightly towards puberty (Colenbrander, van de Wiel and Wensing, 1980).

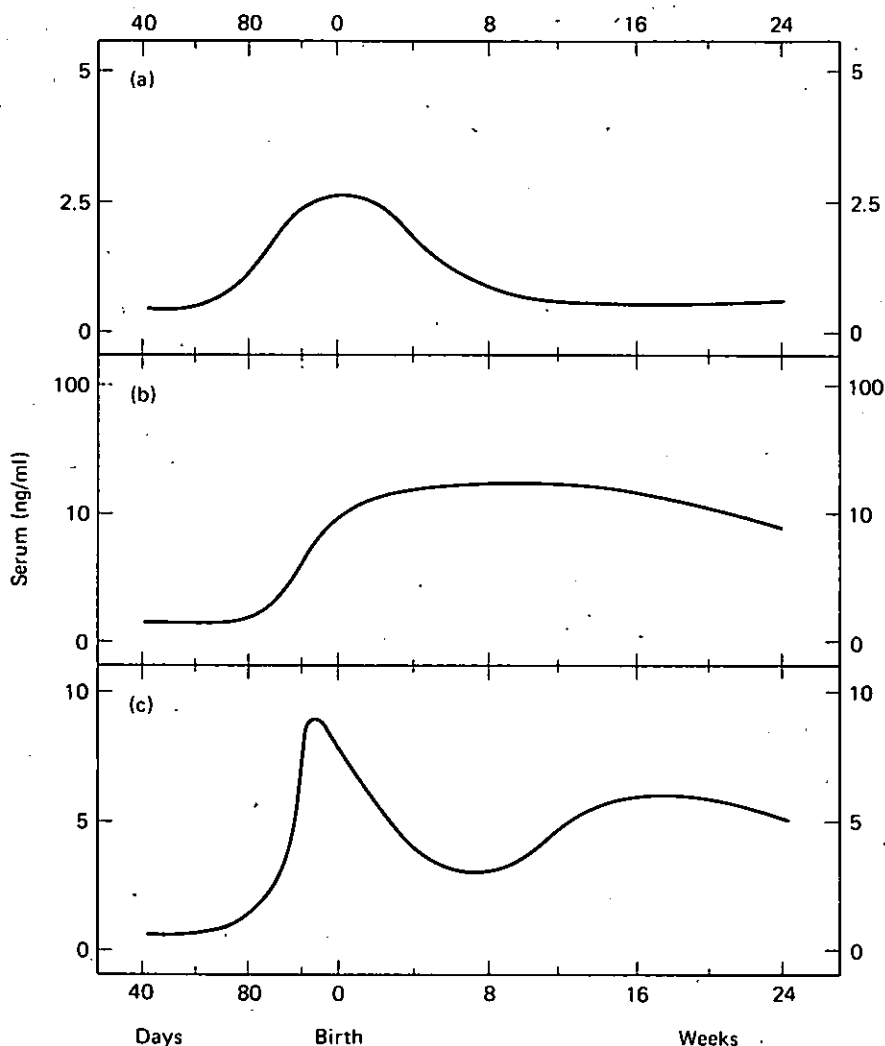


Figure 5.2 Serum levels of (a) LH, (b) FSH and (c) prolactin during sexual development in female pigs. (a) after Elsaesser *et al.* (1976) and Colenbrander *et al.* (1977); (b) after Colenbrander *et al.* (1980); (c) after Colenbrander *et al.* (1981)

Prolactin levels are elevated at birth, decline to a minimum when the gilt is five weeks of age and then increase again (Colenbrander *et al.*, 1981). The physiological importance of this pattern remains speculative, since a definite role for prolactin has thus far not been described in the pig.

As mentioned before, neither changes in hydroxysteroid dehydrogenase activity nor in follicular morphology occur which might be correlated with the period of greater secretion of LH or FSH near the time of birth. This contrasts to the male pig, in which the prenatal rise in serum concentration of FSH is paralleled by marked growth in the length of the seminiferous tubules and an increase in activity of Leydig cells (Colenbrander, van de

Wiel and Wensing, 1980). Further support for the conclusion that early ovarian development, but not testes development, is independent of normal functioning of the pituitary is provided by the observation that foetal decapitation does not interfere with normal germ cell development in the foetus (Colenbrander, Wensing and van Rossum Koh, 1981).

Later phases of follicular growth are, however, regulated by gonadotrophin secretion. The development of tertiary follicles or antral follicles which are first present at eight weeks after birth (Mauleon, 1964; Oxender *et al.*, 1979) may represent a stage when follicles become sensitive to gonadotrophins. Support for the idea that follicular development becomes dependent on gonadotrophins at this stage of development emerges from the observation of the failure of gonadotrophins to cause increased follicular development in gilts of five weeks of age. The critical age at which increased follicular development and ovulations can be induced by exogenous gonadotrophin treatment appears to be about nine weeks (Casida, 1934; Kather and Smidt, 1975; Oxender *et al.*, 1979) and will be discussed elsewhere in this book (Paterson, Chapter 7).

The mechanism by which the pituitary gains control over follicular development in the pig remains to be determined. However, it seems likely that this process is related to the development of gonadotrophin receptors in the ovary, as indicated by studies in the female rat (Siebers *et al.*, 1977). The continuous exposure of the ovary to comparatively high levels of FSH may play a role in this context.

In the mature sow, as in other species, the secretion of LH is pulsatile or episodic in nature and the amplitude and frequency of the LH episodes change during the oestrous cycle (Elsaesser and Parvizi, 1977; Foxcroft, 1978). In view of data from Foster and Ryan (1979) and Wildt, Marshall and Knobil (1980), which stress the importance of the frequency of LH pulses for the onset of puberty, possible maturational changes in the frequency-amplitude characteristics of episodic LH secretion in the gilt are of great interest. In the chronically catheterized foetus, minor fluctuations in LH levels occur during the last week of foetal life (Macdonald *et al.*, 1979). When pigs at 17 days of age are monitored by blood samples taken at two hour intervals, the amplitude of LH discharges appears to increase. This finding might explain the elevated average LH levels at this time (Colenbrander *et al.*, 1977). In gilts at 9–10 weeks of age episodes of LH release occur spontaneously with a mean frequency of 1.3 peaks/hour, but this frequency could be reduced to zero by oestradiol-17 $\beta$  treatment (Figure 5.3; Foxcroft, Pomerantz and Nalbandov, 1975). When profiles of LH levels were based on samples taken at 10 minute intervals, there was a gradual increase in the frequency of LH episodes from prepuberty to late puberty (Stickney and Foxcroft, personal communication), but corresponding data for the period immediately preceding first ovulation are lacking.

It is possible that the postulated increase in frequency of the LH pulses (Foster and Ryan, 1979) occurs in the gilt only during the night period. In gilts of 160 days of age, a significantly greater proportion of raised LH concentrations occurred during the night than during daylight but this difference did not exist in gilts of 60 days of age (Table 5.2; Elsaesser and Foxcroft, 1978). Thus a development in nocturnal LH release appears similar to that of the human, in which sleep-related increments in the

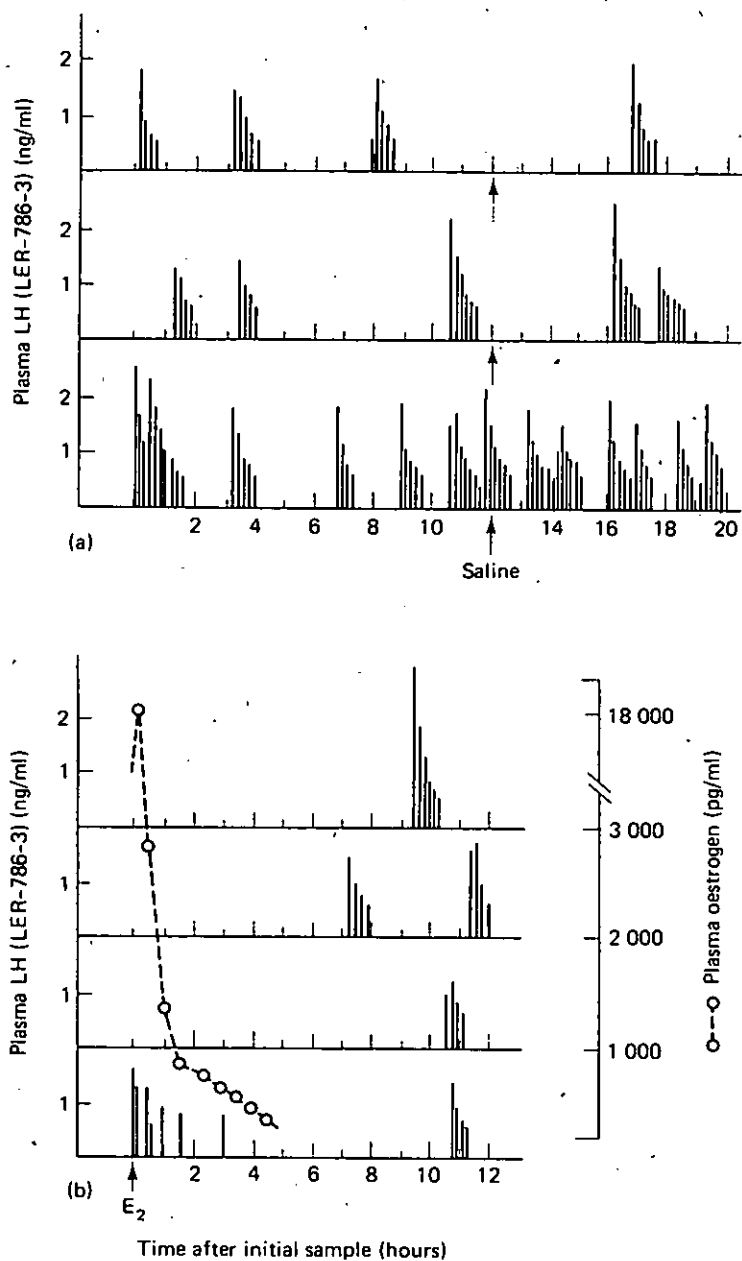


Figure 5.3 (a) Spontaneous episodic release of LH in three untreated prepubertal female pigs. Saline injection given 12 hours after initial sample. Samples were drawn every 10 minutes for LH assay. LH levels not represented by vertical bars were less than 0.7 ng LH/ml. (b) Spontaneous LH release in the 12 hour period following intravenous injection of 5.0 µg oestradiol-17β. Levels of peripheral oestrogens indicated were the means for the same four animals. By courtesy of Foxcroft, Pomerantz and Nalbandov (1975)



**Table 5.2**  $\chi^2$  ANALYSIS (WITH YEATE'S CORRECTION) OF ELEVATIONS IN LH LEVELS FROM INFANTILE AND PERIPUBERTAL GILTS

Age (days)	Nos. LH estimates $\geq 1$ ng/ml plasma		
	per 72 hours	per 3 days	per 3 nights
60 ( $n = 12$ )	82	44	38
160 ( $n = 12$ )	100	33 <sup>(a)</sup>	67 <sup>(a)</sup>

Blood samples were taken at 4 hour intervals for 72 hours.

<sup>(a)</sup>  $P \leq 0.001$ ,  $\chi^2 = 23.8$

From Elsaesser and Foxcroft (1978)

secretion of gonadotrophin have been observed in pubertal children (Weitzman *et al.*, 1975). It may be assumed that the nocturnal increase in number and/or amplitude of LH episodes is associated with the onset of, or an increase in, ovarian oestradiol secretion.

### The responsiveness of the pituitary

The factors responsible for the pattern of plasma gonadotrophin secretion during development in the pig are still poorly understood. The evidence presented, that the ontogeny of LH secretion starts during foetal life, raises the question as to whether the hypothalamus controls pituitary function prior to birth. *In vivo* the pituitary of the 55 day old foetus is unable to respond to a single injection of LH releasing hormone. However, between 70 and 100 days of gestation the proportion of animals responding with a rise in concentrations of LH in the umbilical artery increases and all foetuses older than 100 days are capable of releasing LH with no apparent sex differences (Colenbrander *et al.*, 1980).

Preliminary data obtained *in vitro* demonstrate a similar ontogeny of pituitary responsiveness although in these studies a sex difference was apparent (Figure 5.4). Monolayer cultures of female foetal anterior pituitary cells exposed for four hours to LH releasing hormone had only a slightly increased LH release at 60 days of gestation in the presence of the maximal doses of LH releasing hormone ( $10^{-8}$  M). A typical dose response curve was obtained from pituitaries recovered from foetuses at 80 days of gestation and in cultures from female foetuses at 105 days of gestation both basal LH release and maximal response doubled (Figure 5.4). In both age groups basal and maximal LH release from male pituitary cells and the LH releasing hormone concentration required to produce a half maximal stimulation ( $ED_{50}$ ) of LH release, was lower compared with female pituitary cells (Elsaesser, Bruhn, Parvizi and Heilhecker, unpublished observations). These findings correspond well with the above-mentioned earlier increment of LH levels in female compared with male foetuses and possibly relate to differences in plasma testosterone concentrations.

Evidence to suggest that the foetal hypothalamus is able to release sufficient amounts of LH releasing hormone to enhance pituitary LH secretion is derived from the response to electrical or electrochemical stimulation (Bruhn, Parvizi and Ellendorff, 1981) and will be discussed in detail by Ellendorff and Parvizi in Chapter 9.

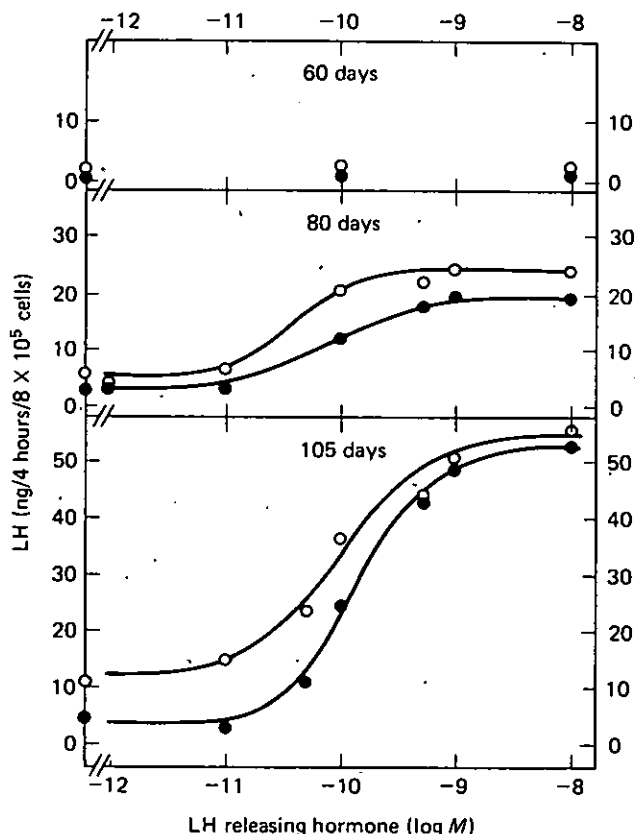


Figure 5.4 Effect of increasing concentrations of LH releasing hormone on LH release by porcine anterior pituitary cells from male (●) and female (○) foetuses. Results are presented as means of data obtained from quadruple monolayer culture dishes. By courtesy of Elsaesser, Bruhn, Parvizi and Heilhecker, unpublished observations

The postnatal development of pituitary responsiveness to LH releasing hormone has not yet been evaluated in the gilt. Currently we are studying this problem by establishing dose response curves to LH releasing hormone in pituitary monolayer cultures and the effect of pretreatment with gonadal steroids. From preliminary results we know that pituitary cells from 60 day old gilts produce basal and maximal LH releases about 20 times greater than those observed from cells of foetuses at 105 days of gestation (Bruhn, Parvizi and Elsaesser, unpublished observations).

### Maturation of negative feedback of gonadal steroids

Developmental changes in the pattern of gonadotrophin secretion may at least in part reflect maturational changes in the feedback mechanisms of the central nervous system-pituitary-gonadal axis. Byrnes and Meyer (1951) and Ramirez and McCann (1965) postulated a maturational change

in the negative feedback action of gonadal steroids. This hypothesis, called the gonadostat theory, has been favoured as an explanation for the mechanisms involved in the onset of puberty. This theory of sexual maturation states that the threshold for negative feedback in the hypothalamo-pituitary unit, the 'gonadostat', increases with age and thereby the sensitivity to the inhibiting effect of sex steroids progressively decreases. This results in increased secretion of gonadotrophin.

Two types of experiments have been performed to demonstrate changes in negative feedback of gonadal steroids in the female pig. One type examines the response to removal of the gonads and the other examines the effect of replacement therapy with gonadal steroids on plasma LH levels.

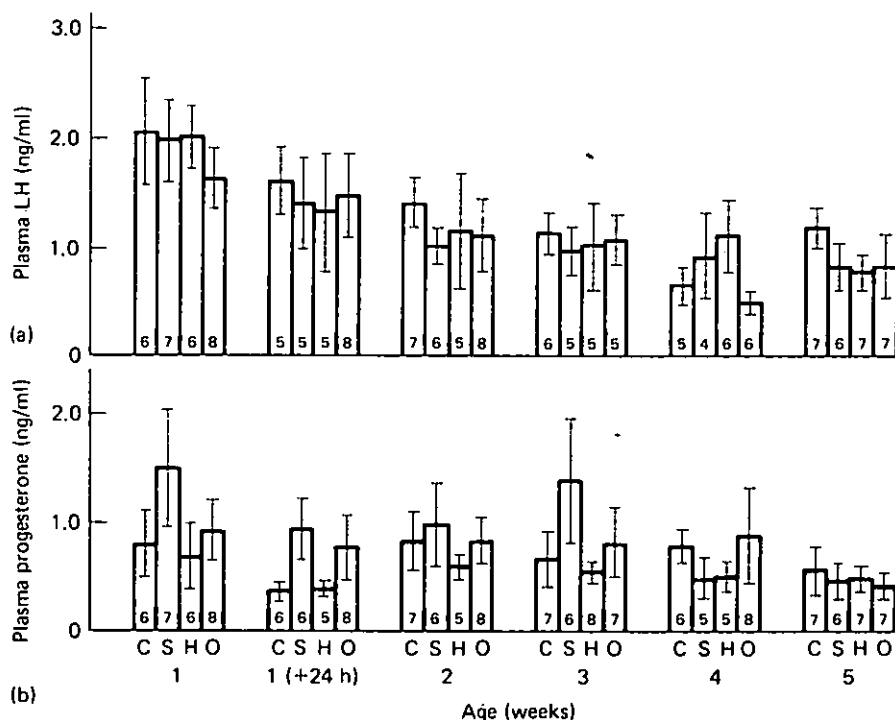


Figure 5.5 Concentrations of (a) LH and (b) progesterone in the plasma of immature female miniature pigs at various ages. Results are means  $\pm$  S.E.M.; the numbers of pigs are given at the base of each column. C—controls; S—sham-operated; H—hemi-ovariectomized; O—ovariectomized (all operations were performed at one week of age). By courtesy of Elsaesser, Parvizi and Ellendorff (1978)

Levels of LH in plasma do not increase during the four weeks following gonadectomy at one week of age in either sex (Figure 5.5, Elsaesser, Parvizi and Ellendorff, 1978). An explanation for the failure of LH to increase after ovariectomy is the possibility that the ovary is producing no steroids. Indeed, the plasma levels of progesterone are generally low and unaffected by treatment and, as noted before, it may be assumed that the ovary is unable to produce oestrogen at this age (Oxender *et al.*, 1979). The

finding that LH decreases between one and five weeks of age in ovariectomized as well as intact animals, indicates that the ovary does not play an important role in the decrease of plasma LH levels occurring at this period of development (Elsaesser, Parvizi and Ellendorff, 1978). While the response to ovariectomy at eight weeks of age has not been tested, the data of Colenbrander *et al.* (1977) are consistent with the view that at this age the hypothalamo-pituitary unit of the male pig has developed its competence to detect and to respond to the removal of the gonads. Increased levels of LH in freemartin pigs (gonads absent) suggest that at eight weeks of age LH release in the female also comes under the control of gonadal secretion. Gilts ovariectomized at either 60 or 130 days of age have

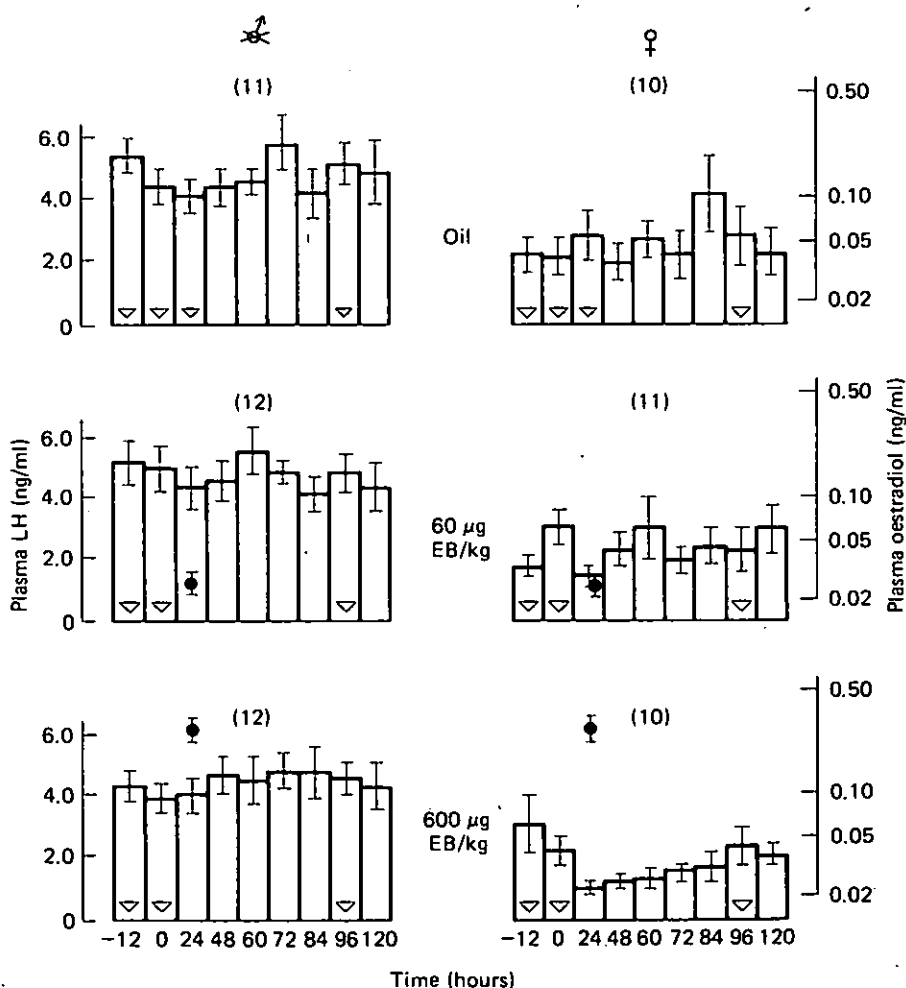


Figure 5.6 Concentrations of LH (open bars) and oestradiol-17β (●, logarithmic scale) in the plasma of orchidectomized male and intact female pigs after treatment with sesame oil or oestradiol benzoate (EB) at six days of age. ▽—plasma concentration of oestradiol-17β ≤ 20 pg/ml. Results are means ± S.E.M.; number of pigs are shown in parentheses. By courtesy of Elsaesser and Parvizi (1979)

elevated LH and FSH levels at 160 days of age (Foxcroft, Stickney and Elsaesser, unpublished observations).

From the effect of exogenous oestradiol on plasma LH it appears that the central nervous system-pituitary unit of the newborn female pig is able to recognize and to respond to changes in concentrations of oestradiol-17 $\beta$ . The negative oestrogen feedback matures earlier in the female than in the male (Figure 5.6; Elsaesser and Parvizi, 1979). However, negative feedback responses can only be evoked by a sustained increase in the concentration of oestradiol-17 $\beta$  in the plasma. This is concluded from the finding that the concentration of LH in plasma of female pigs at six days of age was significantly suppressed 24 hours after the administration of 600  $\mu$ g oestradiol benzoate/kg body weight whereas treatment with 60  $\mu$ g oestradiol benzoate/kg body weight did not affect LH.

As gilts mature, the effectiveness of oestrogen in suppressing the secretion of LH seems to be enhanced. For example, intravenous injections of oestradiol or the subcutaneous implantation of oestradiol in silastic capsules, suppress LH levels in 9–10 week old gilts. These studies also demonstrated that the negative feedback action of oestradiol is exerted through inhibition of spontaneous episodic LH release (Pomerantz, Foxcroft and Nalbandov, 1975; Foxcroft, Pomerantz and Nalbandov, 1975).

The hypothalamus as well as the anterior pituitary may be a site of oestradiol negative feedback action in the immature gilt and nuclear and cytoplasmic receptors for oestradiol and progesterone have been demonstrated in both the hypothalamus and the pituitary. No difference in the number of binding sites was found in gilts between one month and 5.5 months of age. However, it is claimed that the capacity of cytoplasmic binding sites for oestradiol in mature cycling pigs is 15-fold higher than in prepubertal pigs (Diekman and Anderson, 1979).

There are no studies which clearly indicate a decrease in the feedback sensitivity of gonadal steroids at puberty in the gilt. There is evidence that the negative feedback action of testosterone declines during sexual maturation in the boar. In the absence of any dramatic changes in levels of LH, this decline in feedback sensitivity to testosterone may be a compensatory, rather than an initiating mechanism, necessary for the maintenance of LH release in the presence of raised levels of testosterone during puberty (Elsaesser, Parvizi and Ellendorff, 1978). Subtle changes in the amplitude and/or frequency of episodic LH release, however, cannot be excluded.

### **Development of the stimulatory oestrogen feedback mechanism**

Expression of the stimulatory oestrogen feedback mechanism is essential for the onset of cyclic ovarian activity, since it triggers the surge release of LH which in turn induces ovulation.

The capability of the hypothalamo-pituitary unit to respond to an increase in circulating oestrogen levels with a surge of LH develops gradually as the gilt matures. As mentioned before, a single intramuscular injection of 60 or 600  $\mu$ g oestradiol benzoate/kg body weight will not elicit an LH discharge in gilts at seven days of age (Elsaesser and Parvizi, 1979). At 14 days of age, female miniature pigs, given a single dose of oestradiol

benzoate respond with a significant increase in the levels of LH in plasma 60–72 hours later (Figure 5.7; Elsaesser, Parvizi and Ellendorff, 1978). The concentration of oestradiol in plasma must be raised both over a certain period of time and over a certain threshold level to evoke positive response (Yamaji *et al.*, 1971), e.g. an intramuscular injection of 6 µg oestradiol benzoate/kg body weight (Elsaesser, unpublished) does not increase the levels of LH in the plasma of immature miniature gilts.

These observations do not imply that the control system which responds to oestrogen is fully mature in the miniature pig at 14 days of age. In fact, it is evident from a comparison of the characteristics of oestrogen-induced LH surges in 60 and 160 day old domestic gilts, that several maturational changes occur (Figure 5.8; Elsaesser and Foxcroft, 1978). In 60 day old

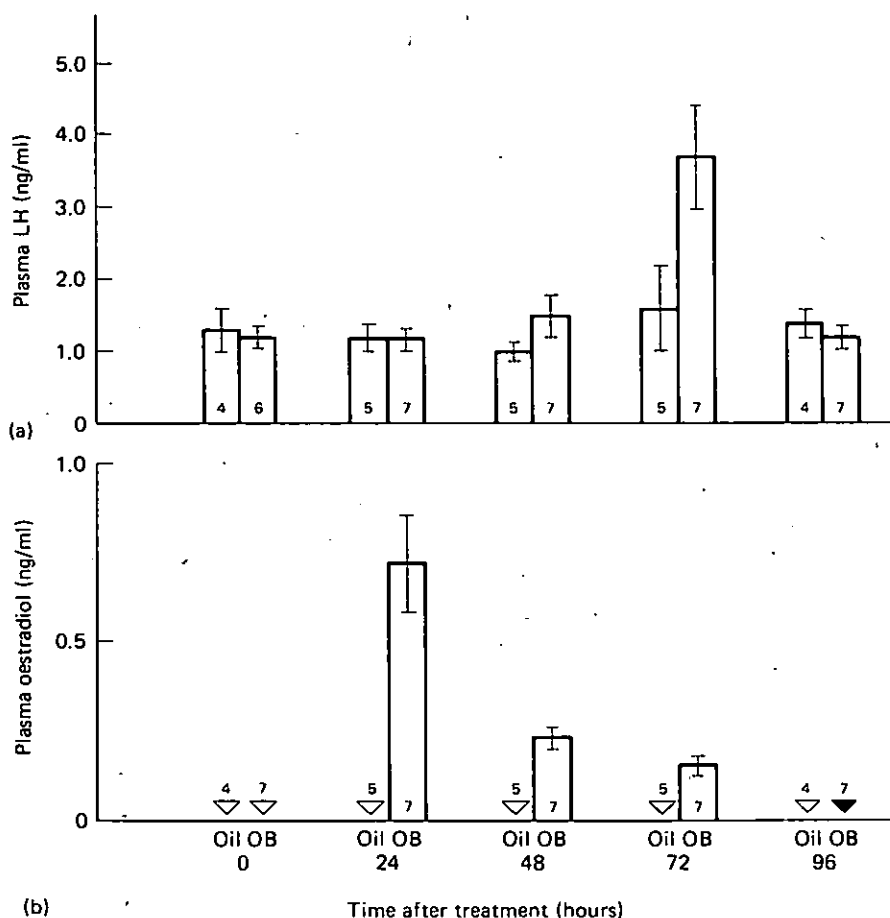


Figure 5.7 Concentrations of (a) LH and (b) oestradiol in plasma of newborn female miniature pigs after treatment with sesame oil or oestradiol benzoate (OB, 0.6 mg/kg body weight) at two weeks of age. ▽—Oestradiol concentration  $\leq 20$  pg/ml plasma; ▼—oestradiol concentration less than 100 pg/ml plasma. Results are means  $\pm$  S.E.M.; numbers of pigs are shown at the base of each column. By courtesy of Elsaesser, Parvizi and Ellendorff (1978)

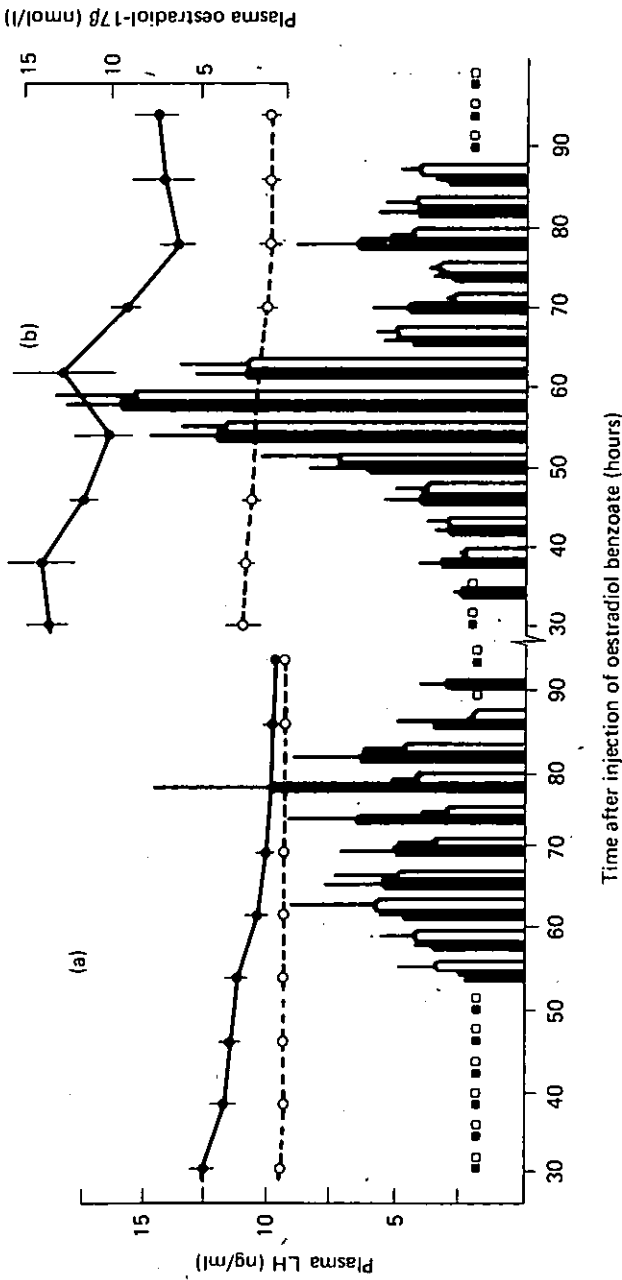


Figure 5.8 Plasma concentrations (means  $\pm$  S.E.M.) of LH (histogram bars) and oestradiol-17 $\beta$  (graph lines) in (a) 60-day old and (b) 161-day old gilts treated with 60  $\mu$ g oestradiol benzoate/kg (broken lines, open bars) or 600  $\mu$ g oestradiol benzoate/kg (solid lines, solid bars).  $\square$ —Concentration of LH below the limit of detection of the assay. By courtesy of Elsaesser and Foxcroft (1978)

gilts the pattern of LH release subsequent to the injection of oestradiol benzoate is more variable and the mean concentrations of LH are lower than in gilts 160 days of age. The pattern of LH release is also less well defined in the younger gilts. It appears that the dose of oestradiol benzoate modifies the profile of the LH surge in younger gilts. Another major maturational change relates to the overall mean times to both the onset of the LH discharge and to the peak level of LH, which are greater in gilts at 60 days of age.

A notable feature of the stimulatory oestrogen feedback action in the immature gilt is the tendency for biphasic and sometimes even triphasic surges with a nocturnal preference (Figure 5.9). This phenomenon has

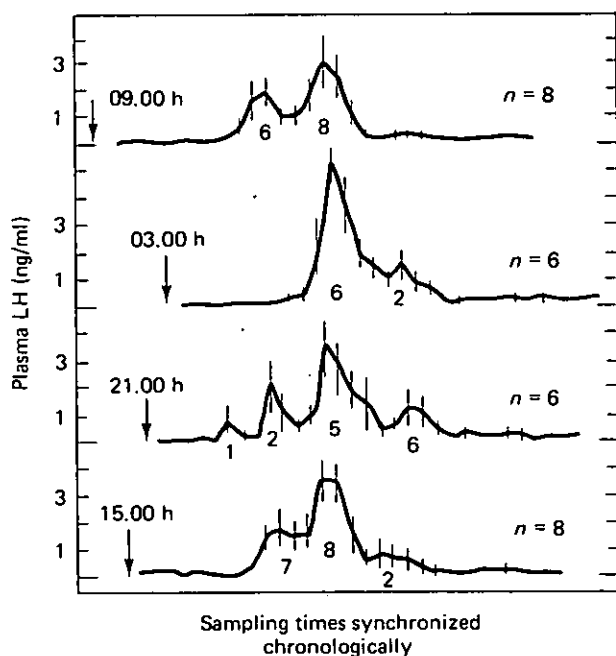


Figure 5.9 Plasma concentrations (means  $\pm$  S.E.M.) of LH in gilts (80 kg) treated with oestradiol benzoate (20  $\mu$ g/kg) at different times of the day ( $\downarrow$ ). From Foxcroft and Piontek (personal communication)

recently been studied in more detail (Foxcroft and Piontek, personal communications) by comparing the gonadotrophin response to the administration of oestradiol benzoate at different times of the day. Both the time to surges, and the number of surges, varied with the time of injection and phases of LH release occurred preferentially during the hours of darkness. A single large LH surge showing the characteristics of the LH surge in mature females was induced when oestradiol was given at 03.00 hours. The authors suggest that a diurnal/nocturnal influence interacts with the LH surge mechanism in the immature gilt. It is of interest that even in the mature cyclic sow the majority of spontaneous surges of LH may be associated with the night and early morning periods (Parvizi *et al.*, 1976); however, following exogenous oestradiol treatment in weaned mature



sows (Edwards, 1980) as well as during late lactation (Elsaesser and Parvizi, 1980), no such synchronization of LH surges to the nocturnal period exists and a single LH discharge is usual, as is the case in the immature lamb (Foster and Karsch, 1975). Thus it seems that either further maturation of the control system which governs the LH discharge occurs, or that the cyclic changes in ovarian hormones present in the mature female predispose the hypothalamo-pituitary unit to a single discharge of LH.

It is not known why the LH surge mechanism is unable to operate soon after birth, nor is it clear what determines its gradual maturation. That the medial preoptic-anterior hypothalamic area may form an essential part of the mechanisms that control ovulation in the pig, has been shown by the induction of cystic ovaries in pigs with lesions in this area (Döcke and Busch, 1974). It is unlikely that the pituitary of the neonate is unable to release LH in a short pulse, because our previous studies suggest that the pituitary of the newborn male and female pig responds to LH releasing hormone in the same way as the adult (Elsaesser *et al.*, 1974). However, although it is possible that the pituitary in these animals is not able to release LH in a surge-like manner similar to the preovulatory surge, this has not been studied so far in the pig. The recent aforementioned finding that the number of cytoplasmic and nuclear oestradiol receptors in the hypothalamus and in the pituitary does not change in the gilt between one and 5.5 months of age (Diekman and Anderson, 1979) would argue against the assumption that changes in specific oestradiol binding sites are involved in the development of oestrogen-induced LH secretion.

With respect to possible causes of the maturation of the stimulatory oestrogen feedback, preliminary unpublished results of Foxcroft, Stickney and Elsaesser suggest that oestrogens are not involved in this process up to 60 days of age, as indicated by the failure to hasten the maturity of the LH surge response to oestrogen in immature gilts, which had been implanted with silastic capsules containing small amounts of oestradiol. In groups of gilts in which the LH response was evaluated at 160 days no further maturation of the positive feedback mechanism occurred in females ovariectomized at 60 days of age. However, substantial maturation was observed in animals either ovariectomized at 130 days or in gilts given implants of oestradiol following ovariectomy at 60 days of age. Ovarian oestrogen appears therefore to have a role in the maturation of the LH surge mechanism.

On the basis of available information, therefore, the LH surge mechanism may be considered essentially mature some time before the onset of ovarian cyclicity. The conclusion can then be drawn that the timing of the first cyclic discharge during puberty is not limited by the capability of the central nervous-pituitary system to respond to the positive feedback action of oestradiol. Rather it appears that before puberty the ovary is unable to release a signal in the form of an oestradiol surge.

#### SEXUAL DIFFERENTIATION OF THE STIMULATORY OESTROGEN FEEDBACK MECHANISM AND THE EFFECT OF PRENATAL TESTOSTERONE TREATMENT

One interesting feature of the stimulatory oestrogen feedback mechanism is its sexual dimorphism in various species, which is a consequence of

testicular androgen secretion during a critical period of foetal or neonatal life (Barracrough, 1966; Gorski, 1973).

In the miniature pig, the LH surge control system is sexually differentiated as early as 14 days of age. Unlike the newborn female (miniature) pig, the newborn male pig is unable to release LH in response to oestrogen (Elsaesser, Parvizi and Ellendorff, 1978). That this sex difference is not due to testicular secretion at the time of oestradiol benzoate treatment is shown by the lack of an LH discharge in 14 day old or 160 day old male castrates. However, subsequent to the negative feedback action of oestradiol, LH levels in 160 day old castrated boars rise to pretreatment levels at 72 hours, although the plasma levels of oestradiol are still elevated (Figure 5.10, Elsaesser and Parvizi, 1979). A similar observation has been made by

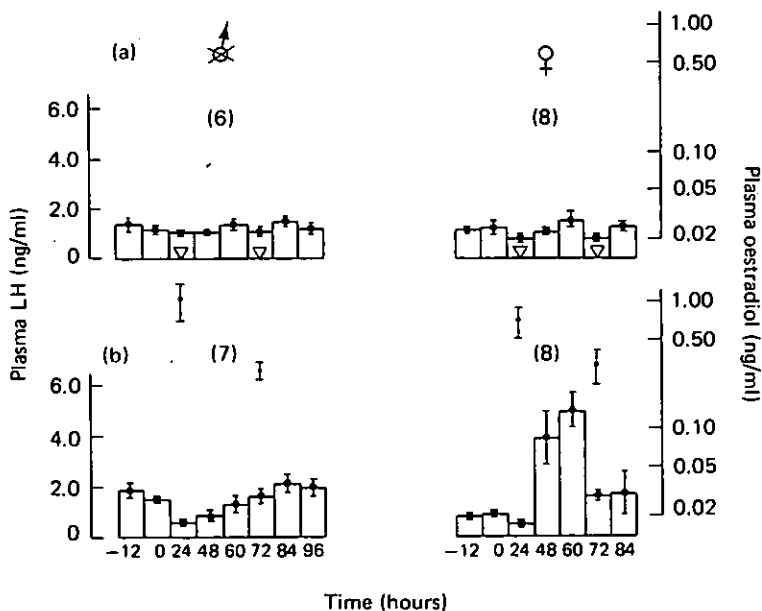


Figure 5.10 Concentrations of LH (open bars) and oestradiol-17 $\beta$  (●, logarithmic scale) in the plasma of orchidectomized male and intact female pigs after treatment with (a) sesame oil, or (b) oestradiol benzoate (60  $\mu$ g/kg) at 160 days of age.  $\nabla$ —plasma concentration of oestradiol-17 $\beta$   $\leq$ 20 pg/ml. Results are means  $\pm$  S.E.M.; numbers of pigs are shown in parentheses. By courtesy of Elsaesser and Parvizi (1979)

Ford and Schanbacher (1977) following daily treatment of castrated boars with oestradiol benzoate, suggesting the possibility that the sexual dimorphism is quantitative rather than qualitative. In this context it is of interest that castrated boars display clear sexual receptive behaviour when treated with oestradiol benzoate.

The findings that the foetal testis increases its secretion of testosterone during differentiation of the internal and external genitalia between days 30 and 50 of foetal life (Colenbrander, de Jong and Wensing, 1978; Raeside and Middleton, 1979; Ford, Christenson and Maurer, 1980) and that neonatal treatment with testosterone is ineffective in inducing an ovulatory sterility (Zimbelman and Lauderdale, 1973), raise the possibility

that a sexual differentiation of the stimulatory oestrogen feedback system occurs early in foetal life.

So far no definite answer can be given to the question of whether the concept of androgen-dependent sexual differentiation of the LH control system is valid for the pig. In prepubertal female offspring exposed to testosterone propionate via their mother (intramuscular injection on three occasions separated by two-day intervals, 5 mg/kg on days 30, 50 or 70 of foetal life) the positive response of LH to oestradiol benzoate was impaired (Figure 5.11) and by 250 days the weight of the ovaries and the uterus was reduced and the number of animals that had ovulated was depressed. Gilts treated with testosterone propionate later during foetal life did not differ from controls in these respects (Elsaesser and Parvizi, 1979).

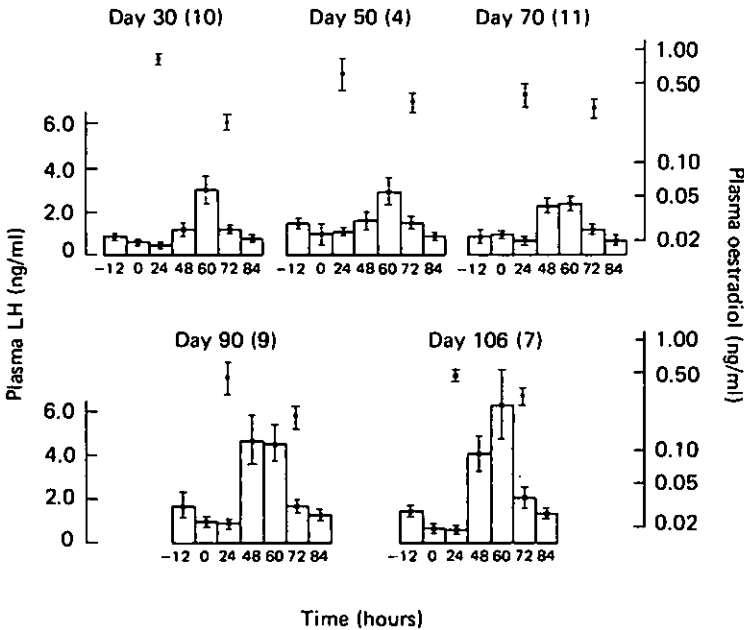


Figure 5.11 Concentrations of LH (open bars) and oestradiol-17 $\beta$  (●, logarithmic scale) in the plasma of intact female pigs after treatment with oestradiol benzoate (60  $\mu$ g/kg) at 160 days of age. The mothers of these animals had been treated with testosterone propionate on days 30, 50, 70, 90 or 106 of pregnancy. Results are means  $\pm$  S.E.M.; numbers of pigs are shown in parentheses. By courtesy of Elsaesser and Parvizi (1979)

The effects of transuterine intrafoetal testosterone treatment support the view that the pig foetus is well protected against the masculinizing effects of maternally administered androgens and that complete suppression of the LH surge mechanism as in the genetic male might be a transient effect.

Clitoral enlargement was observed in females which had been treated intrafoetally with testosterone (20 mg/foetus) on day 40 or 50 of gestation. This treatment also blocked the stimulatory oestrogen feedback mechanism prepubertally and at the time of puberty oestrous behaviour occurred at irregular intervals. Later in life oestrous cycles became more regular,

and plasma progesterone profiles as well as the observation of corpora lutea or corpora albicans at laparoscopy indicated the occurrence of ovulation. In some animals failure to ovulate was suggested by elevated LH levels and the occurrence of follicular cysts. Treatment with testosterone on day 30 of gestation resulted in phenotypically masculinized females with empty scrotal sacs and a penis; the LH control mechanism, however, was not disturbed and at about 300 days of age progesterone profiles indicated normal ovarian activity. This was later confirmed by examining the ovaries at laparoscopy or laparotomy (Elsaesser, Ellendorff and Parvizi, unpublished observations).

Although it is tempting to speculate that the critical period for sexual differentiation of the control of surge LH release occurs in the pig during the second trimester of gestation, the inability to achieve total defeminization of the LH control system, including suppression of regular cycles and ovulation, does not rule out the possibility that the observed effects are pharmacological findings. The notion mentioned above, that ovarian secretion might play a role in the development of the stimulatory oestrogen feedback action in the female, has led us (Colenbrander, Parvizi and Elsaesser, unpublished observations) to compare the competence of male and female pigs which have been castrated early in life and pretreated with low amounts of oestradiol for 10 days to discharge LH. Whereas the LH surge mechanism can be activated by a similar treatment in the orchidectomized rhesus monkey (Karsch, Dierschke and Knobil, 1973), LH levels in the castrated boar rose only slightly, but significantly above pretreatment levels. In gilts ovariectomized at 20 days of age the magnitude of the LH discharge was smaller compared with gilts ovariectomized at 150 days of age. It remains to be determined whether ovariectomy even earlier in life totally abolishes the stimulatory oestrogen feedback action. More direct evidence that ovarian secretions are involved in the sexual differentiation of the LH surge mechanism is difficult to obtain in the pig, since attempts to transplant ovaries to orchidectomized piglets have so far failed.

## Conclusion

It is evident from this review that sexual maturation is associated with changes at all levels of the central nervous system-pituitary-ovarian axis. Early ovarian development appears to be independent of gonadotrophic control, while later phases of ovarian development are regulated by gonadotrophin secretion. The anterior pituitary is active long before birth as indicated by the rise in titres of gonadotrophin and changes in its responsiveness to LH releasing hormone *in vivo* and *in vitro*. Negative feedback control of LH release by ovarian secretions is absent during the first weeks after birth and appears to develop between 5 and 8 weeks of age. The stimulatory oestrogen feedback LH discharge mechanism gradually develops and can be considered essentially mature shortly before the onset of puberty.

Although it is still premature to explain the pubertal process in the female pig, it appears that the onset of puberty is neither limited by the functioning of the stimulatory oestrogen feedback mechanism, nor by the

pituitary or the ovary, as each can be activated prepubertally by treatment with appropriate hormones. Evidence in the pig and in other species suggests that puberty is brought about by a reduction in the intrinsic CNS inhibitory mechanism and/or a decrease in the negative feedback action of gonadal steroids, resulting in stimulation of pulsatile LH releasing hormone release and consequently augmentation of episodic LH secretion. This in turn stimulates ovarian function. In addition to the activation of ovarian steroidogenesis, a maturational decrease in the weight-corrected metabolic clearance rate of oestradiol appears to contribute to the elevation of oestradiol levels and thus to the constitution of an effective oestrogen feedback signal, which triggers the first cyclic discharge of LH.

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## References

- ALLEN, B.M. (1904). The embryonic development of the ovary and testis of the mammals. *Am. J. Anat.* **3**, 89–144
- BARNES, R.J., COMLINE, R.S. and SILVER, M. (1974). Foetal and maternal plasma progesterone concentrations in the sow. *J. Endocr.* **62**, 419–420
- BARRACLOUGH, C.A. (1966). Modifications in the CNS regulation of reproduction after exposure of prepubertal rats to steroid hormones. *Recent Prog. Horm. Res.* **22**, 503–539
- BRUHN, TH., PARVIZI, N. and ELLENDORFF, F. (1981). Ability of the fetal hypothalamus to alter LH-secretion in response to electrical and electrochemical stimulation. *Acta endocr.* **96**, Suppl. **240**, 46
- BYRNES, W.W. and MEYER, R.K. (1951). The inhibition of gonadotrophic hormone secretion by physiological doses of estrogen. *Endocrinology* **48**, 133
- CASIDA, L.E. (1934). Prepubertal development of the pig ovary and its relationship to stimulation with gonadotrophic hormones. *Anat. Rec.* **61**, 389–396
- CHOONG, C.H. and RAESIDE, J.I. (1974). Chemical determination of oestrogen distribution in the foetus and placenta of the domestic pig. *Acta endocr.* **77**, 171–185
- COLENBRANDER, B., de JONG, F.H. and WENSING, C.J.G. (1978). Changes in serum testosterone concentrations in the male pig during development. *J. Reprod. Fert.* **53**, 377–380
- COLENBRANDER, B., van de WIEL, D.F.M. and WENSING, C.J.G. (1980). Changes in serum FSH concentrations during fetal and pre-pubertal development in pigs. *VIIth Int. Congr. Endocr.* Abstract No. 37
- COLENBRANDER, B., WENSING, C.J.G. and van ROSSUM KOH, C.M.J.E. (1981). The decapitated pig fetus as a model for the study of the morphological and functional development of some endocrine organs. *Acta morph. neerl.-scand.* (Abstract, in press).

- COLENBRANDER, B., KRUIP, TH.A.M., DIELEMAN, S.J. and WENSING, C.J.G. (1977). Changes in serum LH concentrations during normal and abnormal sexual development in the pig. *Biol. Reprod.* **17**, 506–513
- COLENBRANDER, B., MacDONALD, A.A., PARVIZI, N. and ELSAESSER, F. (1980). Changing responsiveness of fetal pig pituitary to LHRH. *XXVII Int. Congr. Physiol. Sci.*, Abstract No. 1106
- COLENBRANDER, B., MacDONALD, A.A., MEIJER, J.C., ELLENDORFF, F., van de WIEL, D.F.M. and BEVERS, M.M. (1981). Prolactin in the pig fetus. *Eur. J. Obstet. Gynaec. Reprod. Biol.* (Abstract, in press).
- DIEKMAN, M.A. and ANDERSON, L.L. (1979). Quantitation of nuclear and cytoplasmic receptors for estradiol-17 $\beta$  and progesterone in the pituitary, hypothalamus, and uterus in the gilt during prepubertal development. *Proc. 71st Ann. Meet. Am. Soc. Anim. Sci., University of Arizona, Tucson*, pp. 290–291
- DÖCKE, F. and BUSCH, W. (1974). Evidence for anterior hypothalamic control of cyclic gonadotrophin secretion in female pigs. *Endocrinology* **63**, 415–421
- EDWARDS, S. (1980). Reproductive physiology of the post parturient sow. PhD Thesis. University of Nottingham
- ELSAESSER, F. and FOXCROFT, G.R. (1978). Maturation changes in the characteristics of oestrogen-induced surges of luteinizing hormone in immature domestic gilts. *J. Endocr.* **78**, 455–456
- ELSAESSER, F. and PARVIZI, N. (1977). Prepubertal active immunization against gonadal steroids: effect on estrus, ovulation and the oscillatory pattern of plasma LH and progesterone in the female pig. *Acta endocr.* **84**, Suppl. **208**, 107–108
- ELSAESSER, F. and PARVIZI, N. (1979). Estrogen feedback in the pig: sexual differentiation and the effect of prenatal testosterone treatment. *Biol. Reprod.* **20**, 1187–1193
- ELSAESSER, F. and PARVIZI, N. (1980). Partial recovery of the stimulatory oestrogen feedback action on LH release during late lactation in the pig. *J. Reprod. Fert.* **59**, 63–67
- ELSAESSER, F., PARVIZI, N. and ELLENDORFF, F. (1978). Steroid feedback on luteinizing hormone secretion during sexual maturation in the pig. *J. Endocr.* **78**, 329–342
- ELSAESSER, F., STICKNEY, K. and FOXCROFT, G.R. (1982). A comparison of metabolic clearance rates of oestradiol-17 $\beta$  in immature and peripubertal female pigs and possible implications for the onset of puberty. *Acta endocr.* (in press)
- ELSAESSER, F., ELLENDORFF, F., PARVIZI, N. and KONIG, A. (1974). Response of the pituitary and testes to LHRH in the neonatal miniature pig. *Acta endocr. Suppl.* **184**, 29
- ELSAESSER, F., ELLENDORFF, F., POMERANTZ, D.K., PARVIZI, N. and SMIDT, D. (1976). Plasma levels of luteinizing hormone, progesterone, testosterone and 5 $\alpha$ -dihydrotestosterone in male and female pigs during sexual maturation. *J. Endocr.* **68**, 347–348
- FORD, J.J. and SCHANBACHER, B.D. (1977). Luteinizing hormone secretion and female lordosis behaviour in male pigs. *Endocrinology* **100**, 1033–1038
- FORD, J.J., CHRISTENSON, R.K. and MAURER, R.R. (1980). Serum testosterone concentrations in embryonic and fetal pigs during sexual differentiation. *Biol. Reprod.* **23**, 583–587

- FOSTER, D.L. and KARSCH, F.J. (1975). Development of the mechanism regulating the preovulatory surge of luteinizing hormone in sheep. *Endocrinology* **97**, 1205-1209
- FOSTER, D.L. and RYAN, K.D. (1979). Endocrine mechanisms governing transition into adulthood: a marked decrease in inhibitory feedback action of estradiol on tonic secretion of luteinizing hormone in lamb during puberty. *Endocrinology* **105**, 896-904
- FOXCROFT, G.R. (1978). The development of pituitary gland function. In *Control of Ovulation* (D.B. Crighton, G.R. Foxcroft, N.B. Haynes and G.E. Lamming, Eds.), pp. 117-138. London, Butterworths
- FOXCROFT, G.R., POMERANTZ, D.K. and NALBANDOV, A.V. (1975). Effects of estradiol-17 $\beta$  on LH-RH/FSH-RH-induced, and spontaneous, LH release in prepubertal female pigs. *Endocrinology* **96**, 551-557
- GORSKI, R.A. (1973). Perinatal effects of sex steroids on brain development and function. *Prog. Brain Res.* **39**, 149-163
- KARSCH, F.J., DIERSCHKE, D.J. and KNOBIL, E. (1973). Sexual differentiation of pituitary function: apparent difference between primates and rodents. *Science* **179**, 484-486
- KATHER, L. and SMIDT, D. (1975). Vergleichende Untersuchungen zur ovariellen Reaktion infantiler weiblicher Schweine der Deutschen Landrasse und des Göttinger Miniaturschweines auf gonadotrope Stimulierung. *Zuchthygiene* **10**, 10-15
- LIWSKA, J. (1975). Development of the adenohipophysis in the embryo of the domestic pig. *Folia morph.* **34**, 211-217
- LIWSKA, J. (1978). Ultrastructure of the adenohipophysis in the domestic pig (*Sus scrofa domestica*). Part I: Cells of the pars anterior. *Folia histochem. cytochem.* **16**, 307-314
- MACDONALD, A.A., ELSAESSER, F., PARVIZI, N., HEILHECKER, A., SMIDT, D. and ELLENDORFF, F. (1979). Progesterone, oestradiol-17 $\beta$  and luteinizing hormone concentrations in the blood and amniotic fluid of chronically catheterized pig fetuses. *J. Endocr.* **80**, 14P
- MACDONALD, A.A., COLENBRANDER, B., ELSAESSER, F. and HEILHECKER, A. (1980). Progesterone production by the pig fetus and the response to stimulation by adrenocorticotrophin. *J. Endocr.* **85**, 34-35
- MAULEON, P. (1964). Deroulement de l'ovogenèse comparé chez différents mammifères domestiques. *Proc. IVth Int. Congr. Anim. Reprod. A.I., The Hague*, **2**, 348-354
- MELAMPY, R.M., HENRICKS, D.M. ANDERSON, L.L. CHEN, C.L. and SCHULTZ, J.R. (1966). Pituitary follicle-stimulating hormone and luteinizing hormone concentrations in pregnant and lactating pigs. *Endocrinology* **78**, 801-804
- OXENDER, W.D., COLENBRANDER, B., van de WEIL, D.F.M. and WENSING, C.J.G. (1979). Ovarian development in fetal and prepubertal pigs. *Biol. Reprod.* **21**, 715-721
- PARVIZI, N., ELSAESSER, F., SMIDT, D. and ELLENDORFF, F. (1976). Plasma luteinizing hormone and progesterone in the adult female pig during the oestrous cycle, late pregnancy and lactation and after ovariectomy and pentobarbitone treatment. *J. Endocr.* **69**, 193-203
- POMERANTZ, D.K., FOXCROFT, G.R. and NALBANDOV, A.V. (1975). Acute and chronic estradiol-17 $\beta$  inhibition of LH release in prepubertal female pigs: time course and site of action. *Endocrinology* **96**, 558-563

- RAESIDE, J.I. and MIDDLETON, A.T. (1979). Development of testosterone secretion in the fetal pig testis. *Biol. Reprod.* **21**, 985-989
- RAMIREZ, V.D. and McCANN, S.M. (1965). Inhibitory effect of testosterone on luteinizing hormone secretion in immature and adult rats. *Endocrinology* **76**, 412-417
- SIEBERS, J.W., PETERS, F., ZENZES, M.T. SCHMIDTKE, J. and ENGEL, W. (1977). Binding of human chorionic gonadotrophin to rat ovary during development. *J. Endocr.* **73**, 491-496
- SMITH, P.E. and DORTZBACH, C. (1929). The first appearance in anterior pituitary of the developing pig foetus of detectable amounts of the hormones stimulating ovarian maturity and general body growth. *Anat. Rec.* **43**, 277-297
- STICKNEY, K. (1982). The physiology of oestrogen-induced puberty in the gilt. PhD Thesis, University of Nottingham
- WEITZMANN, E.D., BOYAR, R.M., KAPEN, S. and HELLMAN, L. (1975). The relationship of sleep and sleep stages to neuroendocrine secretion and biological rhythms in man. *Recent Prog. Horm. Res.* **31**, 399-441
- WILDT, L., MARSHALL, G. and KNOBIL, E. (1980). Experimental induction of puberty in the infantile female rhesus monkey. *Science* **207**, 1373-1375
- YAMAJI, T., DIERSCHKE, D.J., HOTCHKISS, J., BHATTACHARYA, A.N., SURVE, A.H. and KNOBIL, E. (1971). Estrogen induction of LH release in the rhesus monkey. *Endocrinology* **89**, 1034-1041
- ZIMBELMAN, R.G. and LAUDERDALE, J.W. (1973). Failure of prepartum or neonatal steroid injections to cause infertility in heifers, gilts and bitches. *Biol. Reprod.* **8**, 388-391