Maturation of ovarian follicles in the prepubertal gilt

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Summary. The processes of follicle development and puberty are closely related, and both are associated with maturation of the hypothalamic–pituitary–ovarian axis. Prenatal development of the ovary is independent of gonadotrophic stimulation. Beyond 60 days of age (postnatally), tertiary follicles develop and gonadotrophins begin to influence ovarian follicular development. Negative feedback regulation of pituitary gonadotrophins by ovarian secretions develops between 60 and 100 days of age. In the prepubertal gilt, no consistent changes in peripheral FSH, oestrogen or progesterone concentrations have been identified which are associated with recruitment of the first set of preovulatory follicles. Whether LH secretion increases before this recruitment remains equivocal. Few details are available on how gonadotrophic hormones stimulate ovarian function in the prepubertal gilt. On the basis of a follicular maturation model that has been described for the rat, the actions of FSH, LH and oestrogens on follicular cell receptors and the regulation of aromatase activity seem paramount. Aromatization of androgens to oestrogens has been proposed as a central regulator for follicular maturation.

In the prepubertal gilt, a selective increase in peripheral FSH concentration occurs on Day 1 after unilateral ovariectomy, followed by significant increases in ovarian venous concentrations of oestradiol and inhibin on Days 2 and 4 and compensatory growth as measured by follicular fluid volume on Days 2, 4 and 8. Administration of pig follicular fluid to the prepubertal gilt during and after unilateral ovariectomy suppresses compensatory ovarian hypertrophy by mechanisms yet to be determined. In pigs a number of intraovarian factors have been identified, but there is little information on how these factors regulate follicular recruitment and growth. The factor(s) that prevents ovarian follicles in the prepubertal gilt from progressing to ovulation after acquiring the ability to ovulate in response to exogenous gonadotrophins remains unknown.

Introduction

Reproductive activity and associated phenomena in female mammals are first exhibited during a discrete developmental period known as puberty. To more fully understand follicle development and the pubertal process, one must recall that follicle development and puberty are a series of ongoing changes which begin at conception, proceed through gestation and postnatal development and culminate in the ability to reproduce. Each change is intricately dependent on preceding events and is required for succeeding changes. In this review an effort is made to describe a 'follicle development model' for the gilt; and then to present supportive information on (1) general anatomical development of the ovary and follicle formation, (2) hormonal profiles of gilts during fetal and postnatal development, (3) the influence of exogenous factors on follicle maturation in the gilt, and (4) steroidal and non-steroidal factors associated with follicle development. Further study

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of these factors is required to understand fully ovarian follicle maturation and attainment of puberty in gilts. Follicular development depends on the interplay of anatomical development and several control centres which reside outside the ovary (mainly in the hypothalamus and pituitary but also involving other areas of the central nervous system, and the adrenals, thymus, uterus) or within the ovary and the follicle itself.

A model for follicular development in the gilt

A combination of factors is associated with follicular and pubertal development in the female pig. Pubertal development is influenced by several external factors such as the pig's physical environment and social interactions, as well as genetic factors (Wiggins, Casida & Grummer, 1950; Christenson & Ford, 1979; Hemsworth, Cronin & Hansen, 1982). Presently, there is limited information on the mechanisms by which genetic and external environmental factors influence rate of pubertal development. Direct genetic control of the developmental processes is obvious. However, as an animal matures, the genetic control becomes much more complex, and the interaction of the hypothalamic–pituitary–ovarian axis predominates.

A model for prepubertal follicular development that has evolved from studies primarily with rats (Richards, 1975, 1979; Richards, Rao & Ireland, 1978) is briefly described. Before tertiary follicle formation, it appears that granulosa cells start with a small complement of FSH and oestradiol receptors. As the number of granulosa cells increases in proliferating follicles, the number of FSH and oestrogen receptors increases and secretion of small amounts of oestrogen results (Richards *et al.*, 1976). As this proliferative process advances and tertiary follicles containing theca interna cells develop, the cellular components necessary to the current hypothesis (two-cell theory) for follicular oestrogen production are present (Armstrong, Goff & Dorrington, 1979; Leung & Armstrong, 1980). Oestradiol synthesis and secretion require LH to initiate androgen production by the theca interna cells and FSH to stimulate the aromatase activity of granulosa cells converting androgens to oestrogens. Specific sequential changes in the response of granulosa cells to gonadotrophins appear to be related, in part, to hormone-specific regulation of hormone receptors as reviewed by Foxcroft & Hunter (1985). Applicability of this model for pigs has not been fully confirmed, but cultured theca and granulosa cells from prepubertal gilts stimulated with exogenous gonadotrophins do produce steroids in a manner consistent with the two-cell theory. In addition, pig theca cells also produce significant quantities of oestradiol (Evans, Dobias, King & Armstrong, 1981). These in-vitro observations are supported by changes in steroid concentrations of pig follicular fluid during development of preovulatory follicles (Eiler & Nalbandov, 1977; Ainsworth, Tsang, Downey, Marcus & Armstrong, 1980; Channing *et al.*, 1982).

General anatomical development of the ovary and follicle formation

The embryonic gonad, or gonadal ridge, in the pig embryo is first recognizable at 24–26 days *post coitum* (*p.c.*). However, primordial germ cells are noted as early as 18 days *p.c.* in the region of the germinal ridge (Black & Erickson, 1968). From their extragonadal origin, the primordial germ cells migrate from the yolk sac (where they are first noted), to the gut mesentery, then to the coelomic angles and finally to the germinal ridge area (Witschi, 1948; Mintz, 1957). Allen (1904) and Mauléon (1964) suggested that at about 31 or 32 days *p.c.* the fetal gonad in female pigs is differentiated to an ovary which contains egg nests. Meiosis begins as early as Day 40 and is a common characteristic of most ovaries by Day 50 *p.c.* (Fig. 1; Black & Erickson, 1968).

The resting stage of pig oocytes (diplotene) is first recognized at 50 days *p.c.* and 99% of all germ cells are at the diplotene stage by 20 days *post partum*. The number of germ cells increases dramatically from 5000 at 20 days *p.c.* to a peak of 1 100 000 at 50 days *p.c.* (Black & Erickson,
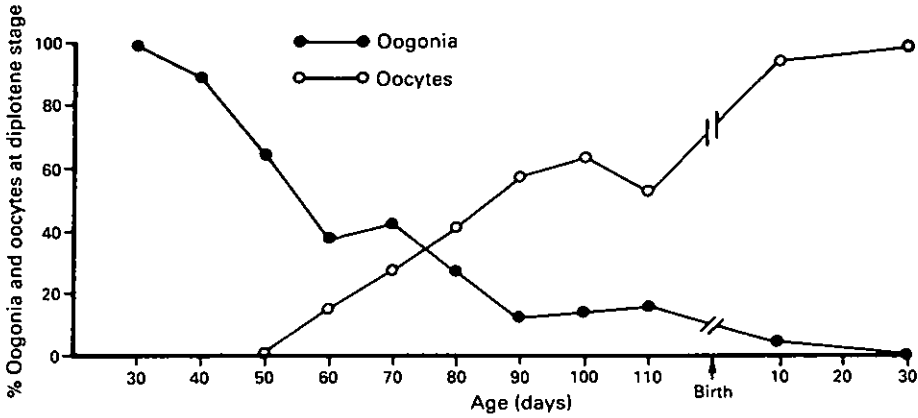
Fig. 1. Distribution of oogonia and oocytes in developing gilts. (After Black & Erickson, 1968.)
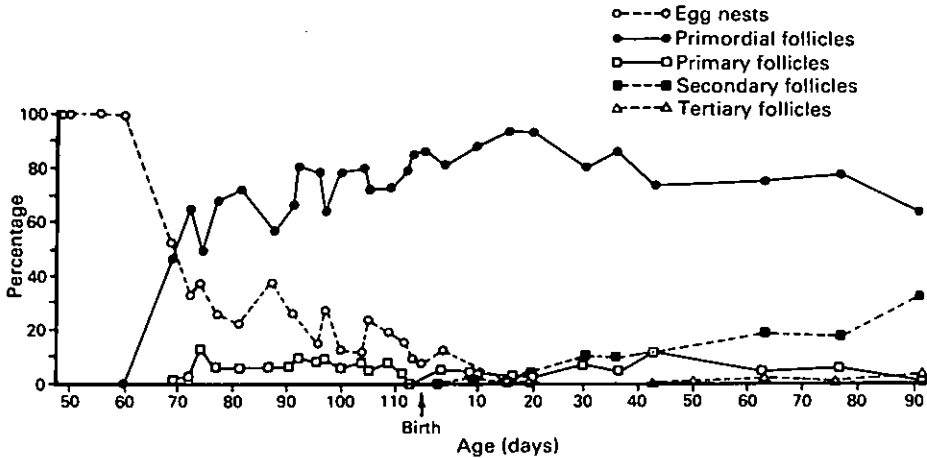

Fig. 2. The number of oogenic structures in ovarian sections from pigs 49 days *post coitum* to 92 days *post partum* graphed as a percentage of the total. (From Oxender *et al.*, 1979.)

1968). Subsequently, germinal mitotic activity decreases and necrosis of germ cells concurrently increases, resulting in a population of about 500 000 germ cells at birth.

All germ cells appear to be contained in egg nests until about 60 days *p.c.* at which time the first primordial follicles are observed (Mauléon, 1964; Oxender, Colenbrander, van de Wiel & Wensing, 1979). The percentages of primordial follicles and egg nests are nearly equal at Day 70 *p.c.* (Fig. 2; Oxender *et al.*, 1979). Thereafter, the percentage of egg nests observed in the ovary decreases as fetal age increases and egg nests are seldom observed in ovaries from pigs 20 or more days after birth. Primordial follicles account for about 80% of the ovarian follicles from 95 days *p.c.* to 90 days after birth. The first primary follicles appear in ovaries of fetuses at 70 days *p.c.*, whereas secondary follicles are initially observed about the time of birth, and the percentage of secondary follicles increases steadily to nearly 30% by 90 days after birth. Tertiary follicles are rarely observed in pigs younger than 60 days of age, and one or more tertiary follicles are observed in only a few of the ovaries from pigs of 60–90 days of age (Oxender *et al.*, 1979). The number of tertiary follicles reaches a peak at about 130 days of age but a large percentage (67%) are atretic (Greenwald, 1978).
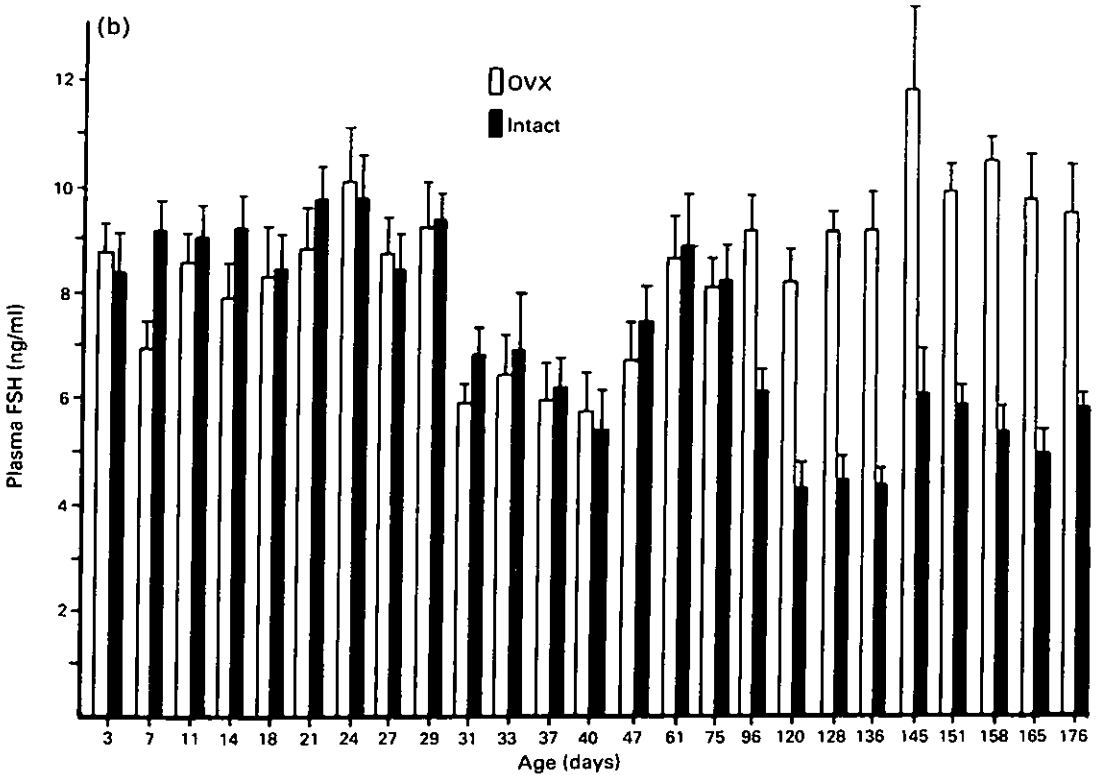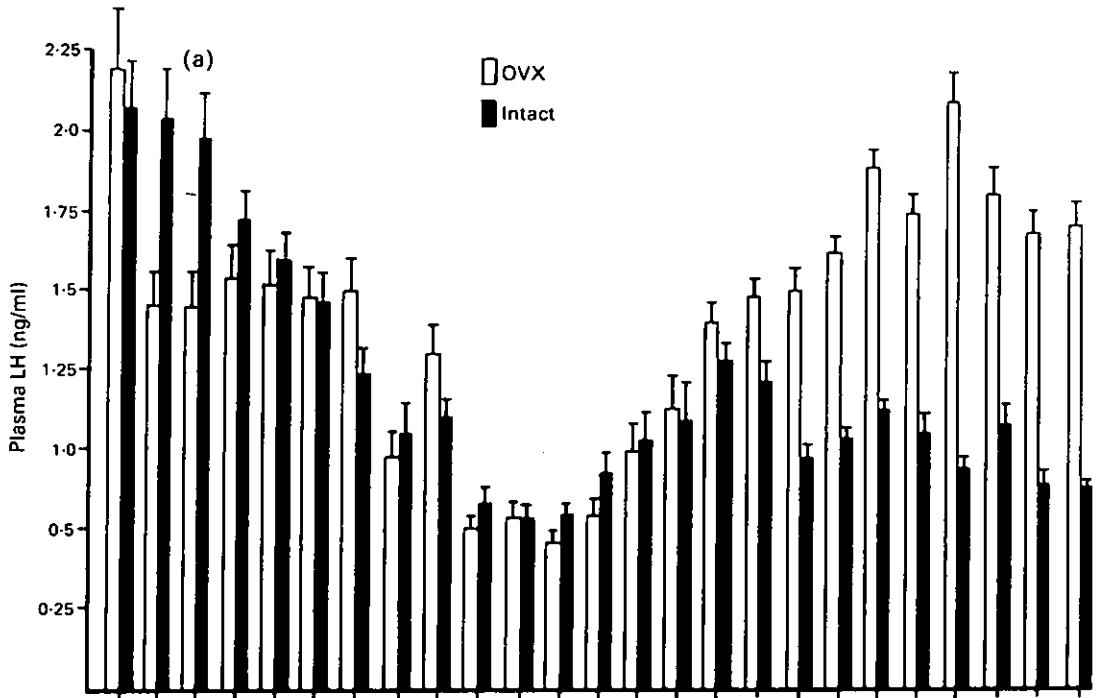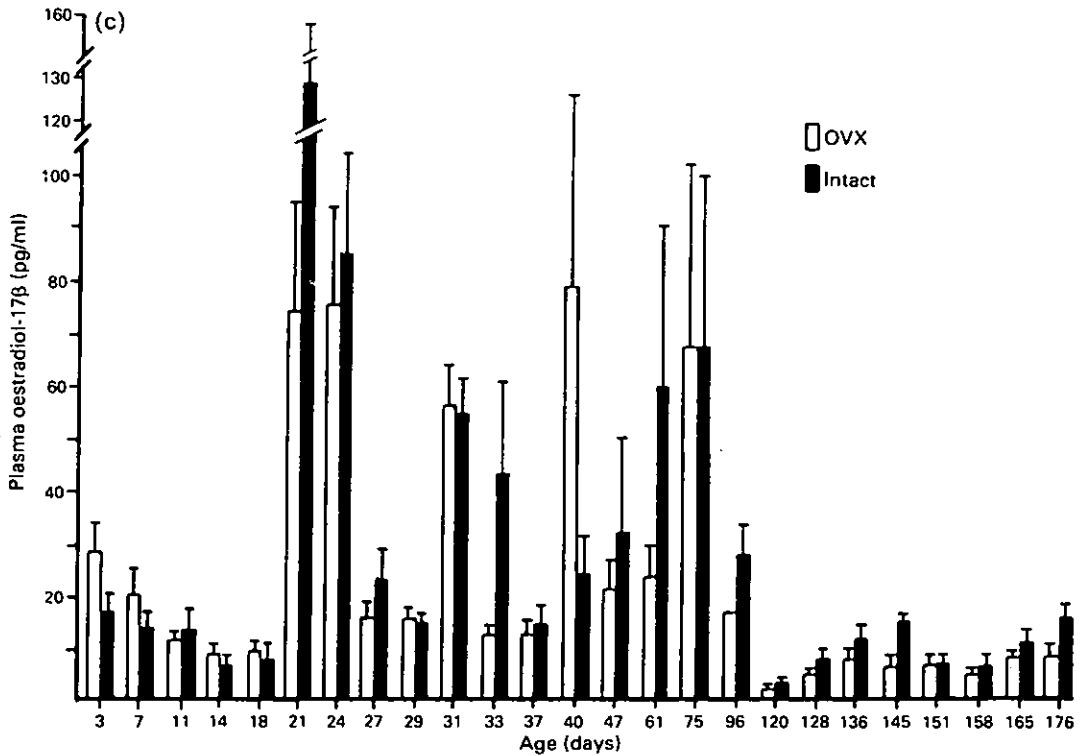
Fig. 3. Plasma concentrations (mean ± s.e.m.) of (a) LH, (b) FSH and (c) oestradiol in ovariec-tomized (OVX) and intact gilts during development. (From Wise, 1982.)

Hormone profiles of gilts during fetal and postnatal development

LH concentrations are low or undetectable before Day 80 of gestation, increase during the last few weeks of fetal life, and remain elevated until birth (Elsaesser, Ellendorff, Pomerantz, Parvizi & Smidt, 1976; Colenbrander, Kruip, Dieleman & Wensing, 1977). FSH concentrations are low before Day 80 of gestation, but after Day 80 they increase sharply in female fetuses and then remain elevated until birth (Colenbrander, van de Wiel, van Rossum-Kok & Wensing, 1982b).

Conjugated and unconjugated oestrogen concentrations in fetal blood are similar in both sexes (Ford, Christenson & Maurer, 1979). Oestrogen concentrations are low at Days 50–60 *p.c.* but increase 15- to 20-fold by Day 95. After an initial oestrogen peak at about 30 days of gestation, oestrogen concentrations in amniotic and allantoic fluid (Ford *et al.*, 1979) and maternal serum (Robertson & King, 1974) increase dramatically from Day 60 *p.c.* to parturition.

Gonadal weight in the female pig gradually increases during fetal development, apparently without any influence from gonadotrophic hormones (Colenbrander, van Rossum-Kok, Oxender & Wensing, 1983). The increase in ovarian weight during fetal development occurs in control fetuses and in fetuses decapitated at 42 days *p.c.* In contrast, gonadal development is disturbed after fetal decapitation of rats (Creasy & Jost, 1966) and male pigs at 42 days *p.c.* (Colenbrander, van Rossum-Kok, van Straaten & Wensing, 1979). In the fetal pig, the pituitary begins to differentiate after day 50 *p.c.* (Danchin & Dubois, 1982; Dacheux & Martinat, 1983) and continues to differentiate from 70 to 110 days *p.c.* (Liwska, 1975; Meijer, Colenbrander, Poot & Wensing, 1985). This increase in the volume and number of gonadotrophic cells is correlated to an increase in pituitary LH and FSH content (Hennen *et al.*, 1982), an increase in responsiveness of LH and FSH secretion

after LHRH (Colenbrander, Macdonald, Elsaesser, Parvizi & van de Wiel, 1982a; Elsaesser, 1982) and an increase in gonadotrophin release in response to electrical stimulation of the hypothalamus (Bruhn, Parvizi & Ellendorff, 1983). However, this increased pituitary activity is not paralleled by a marked increase in ovarian growth (Colenbrander *et al.*, 1977, 1982b).

After birth, the patterns of LH and FSH secretion diverge (Colenbrander *et al.*, 1977; Elsaesser *et al.*, 1976; Elsaesser, Parvizi & Ellendorff, 1978; Wise, 1982). The elevated LH concentration before birth decreases gradually during the first 30 days of life and then remains relatively constant throughout the remaining prepubertal period (Fig. 3a). In contrast to LH, serum FSH concentrations (Fig. 3b) that are elevated at birth remain relatively constant to about 75 days of age and then decline slightly before puberty (Colenbrander *et al.*, 1982a; Wise, 1982; Guthrie, Pursel, Bolt & Nachman, 1984). Serum FSH concentrations in the perinatal period show much less variation than do serum LH concentrations (Colenbrander *et al.*, 1977; Wise, 1982).

A controversy exists as to whether the concentration of LH increases before the onset of puberty in the gilt. If the 'gonadostat' hypothesis for the onset of puberty and first ovulation in the lamb (Foster & Ryan, 1979; Ryan & Foster, 1980) and the heifer (Day *et al.*, 1982) can be applied to the gilt, there should be an increase in serum gonadotrophin concentration before puberty in the face of constant or elevated serum oestrogen (Foster & Ryan, 1979). Studies by Wise (1982), Esbenshade, Paterson, Cantley & Day (1982), Diekman, Trout & Anderson (1983), and Camous, Prunier & Pelletier (1985) did not find any changes in serum LH profiles, or in FSH and prolactin profiles, as gilts approached puberty and first ovulation. In contrast, Pelletier, Carrez-Camous & Theiry (1981), Elsaesser & Foxcroft (1978) and Lutz, Rampacek, Kraeling & Pinkert (1984) have reported increased LH secretion (increased frequency and lower amplitudes) immediately preceding puberty and first ovulation in gilts.

The precise time and mechanism by which the pituitary gonadotrophins begin to influence ovarian and follicular development in the pig remain to be determined. The development of tertiary follicles, beginning about 60 days after birth (Mauléon, 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 following observations. Exogenous gonadotrophins are ineffective in stimulating ovarian follicular development in 30-day-old gilts (Casida, 1935; Oxender *et al.*, 1979) and in some 60-day-old gilts (Kather & Smidt, 1975; Oxender *et al.*, 1979). Oxender *et al.* (1979) reported that 2 of 4 gilts ovulated in response to PMSG (30 i.u./kg body weight) treatment on Day 62 and 65 followed by hCG (30 i.u./kg body weight) on Day 70 of age. Treatment at 100 days of age and older with PMSG and hCG induces follicular growth and ovulation in a high percentage of gilts (Paterson, 1982).

At about 100 days of age, FSH and LH concentrations decrease in intact gilts relative to ovariectomized gilts (Fig. 3). This is indicative of maturation of the negative feedback regulation of pituitary gonadotrophins by ovarian steroids (Wise, 1982). It seems likely that, during this period of maturation, the ovary of the gilt develops gonadotrophin receptors as indicated by studies in the female rat (Siebers, Peters, Zenzes, Schmidtke & Engel, 1977) and the hypothalamic–pituitary axis responds to the presence of the ovaries.

Oestradiol concentrations decrease rapidly after birth (Elsaesser & Foxcroft, 1978; Elsaesser & Parvizi, 1979; Wise, 1982). Wise (1982) reported that concentrations of oestradiol were about 10–20 pg/ml except from Days 20 to 100 when sporadic elevations were observed in intact and ovariectomized gilts (Fig. 3c). At present, the source of this oestradiol is unknown and Elsaesser & Foxcroft (1978) did not observe these elevations in 60-day-old gilts. Both studies are in agreement that oestradiol concentration is low during the remaining prepubertal period. It is not until puberty, or immediately before first ovulation, that oestradiol concentration begins to rise (Karlbom, Einarsson & Edqvist, 1982; Esbenshade *et al.*, 1982). Camous *et al.* (1985) observed that urinary excretion of oestrogen increased 4-fold from 40 to 200 days of age in prepubertal gilts. This suggests that assays of plasma or serum concentrations of oestradiol may lack sensitivity to detect changes in ovarian production during this time.

Plasma progesterone concentrations are low from birth to first ovulation (Elsaesser *et al.*, 1976, 1978; Karlbom *et al.*, 1982; Esbenshade *et al.*, 1982; Wise, 1982). The source of progesterone during prepubertal development is not fully documented but a majority of the steroid is probably of adrenal origin. It appears that the initiation of growth of ovulatory follicles and first oestrus are not a consequence of progesterone secretion (Esbenshade *et al.*, 1982).

Influence of exogenous factors on follicle development in the prepubertal gilt

Casida (1935) demonstrated that exogenous gonadotrophins would induce ovulation in the prepubertal gilt. Paterson (1982) reviewed the use of various dosages of PMSG and hCG in prepubertal gilts and concluded that a useful method for controlled induction of puberty, first ovulation and continuation of oestrous cycles has not yet been obtained.

In spite of the relatively low concentration of gonadal steroids in the prepubertal gilt, many experiments have implied that oestrogen may be involved in the process of follicle development. Meyer & Bradbury (1960) reported that stilboestrol priming markedly augmented the ovarian response to LH in intact and hypophysectomized rats. In the mouse, puberty and first ovulation are induced by the male but the effect can also be produced by exogenous oestrogen therapy if plasma oestradiol is elevated for at least 2 days (Bronson, 1975). Whether this is achieved by the presence of the male, the use of exogenous oestrogen treatment or by a combination of the two, precocious puberty will result. Such findings with mice suggest that exogenous oestradiol should stimulate follicular development and initiate puberty in gilts. However, studies by Dziuk (1965), Baker & Downey (1975), Hughes & Cole (1978) and Paterson, Cantley, Esbenshade & Day (1983) have not conclusively confirmed this effect. Possible explanations for their limited success are dosage of oestrogen, insufficient duration of oestrogen treatment and seasonal effects in response to oestrogen treatment (Paterson, 1982).

An increase in the concentration of endogenous oestrogens may be achieved by an alternative approach. Immunization of animals against oestrogens does not always decrease oestrogens but may, in certain circumstances, increase the concentration of oestrogen at the target tissues. In 1975, Cole reviewed the concept of progonadotrophic and anti-gonadotrophic effects of hCG antisera in animals. Cole, Dewey, Geschwind & Chapman (1975) suggested that small amounts of anti-hCG
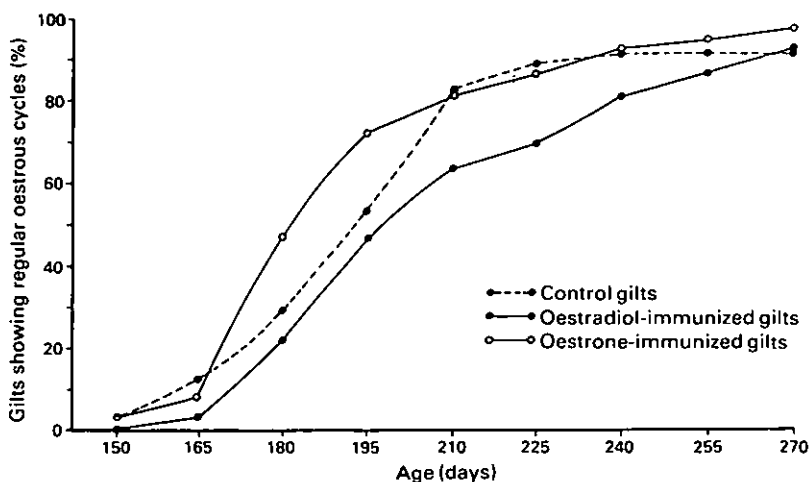

Fig. 4. Oestrous activity or pubertal development relative to age in oestradiol- and oestrone-immunized gilts (After Christenson & Wise, 1985.)

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provoked an enhanced response to gonadotrophins whereas large amounts of anti-hCG inhibited the response. Therefore, a 'pro-steroidal' response may also be achieved through active immunization that produces a low (1:100) antiserum titre (Wise & Schanbacher, 1983; Wise & Ferrell, 1984): with low titre (1:100) systemic binding to oestradiol in heifers there were increased ovulatory follicle numbers, ovarian and uterine weight, and lean body growth. Christenson & Wise (1985) have produced low (1:100) systemic binding to oestradiol and oestrone in developing gilts. Oestradiol and oestrone concentrations are elevated in oestradiol-immunized gilts but remain < 10 pg/ml for control and oestrone-immunized gilts. However, oestrous activity and onset of puberty (first ovulation) appear to be advanced in oestrone-immunized gilts (Fig. 4).

Steroidal and non-steroidal factors associated with follicle development

Studies of members of several mammalian species have shown that the steroid content of follicular fluid changes markedly during development of ovarian follicles, particularly during preovulatory growth (Edwards, 1974; Eiler & Nalbandov, 1977; Goff & Henderson, 1979; Ainsworth *et al.*, 1980). In PMSG/hCG-primed prepubertal gilts, follicle growth increases progressively as determined by follicular diameter (Ainsworth *et al.*, 1980). Follicle growth and development are associated with increasing concentrations of oestrogens and progesterone in follicular fluid during 24–72 h after PMSG treatment (Fig. 5). Upon injection of hCG, the concentration of oestrogens declines rapidly and remains low, whereas the concentration of progesterone drops and then increases rapidly about 6 h before the time of expected ovulation. The concentrations of androgens in follicular fluid increase only slightly after PMSG; however, if changes in follicle size are considered, follicular fluid content of androgens per follicle also increases progressively. The concentrations of oestrogens, androgens and progesterone shown in Fig. 5 are similar to those
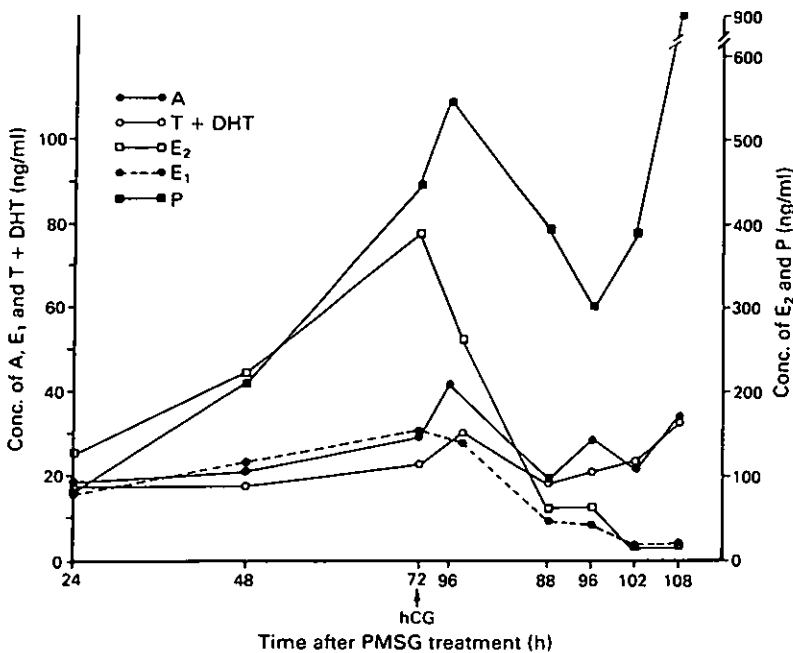

Fig. 5. Follicular fluid concentration of androstenedione (A), testosterone + 5α-dihydrotestosterone (T + DHT), oestradiol (E_2), oestrone (E_1), and progesterone (P) in PMSG/hCG-primed prepubertal gilts. (After Ainsworth *et al.*, 1980.)
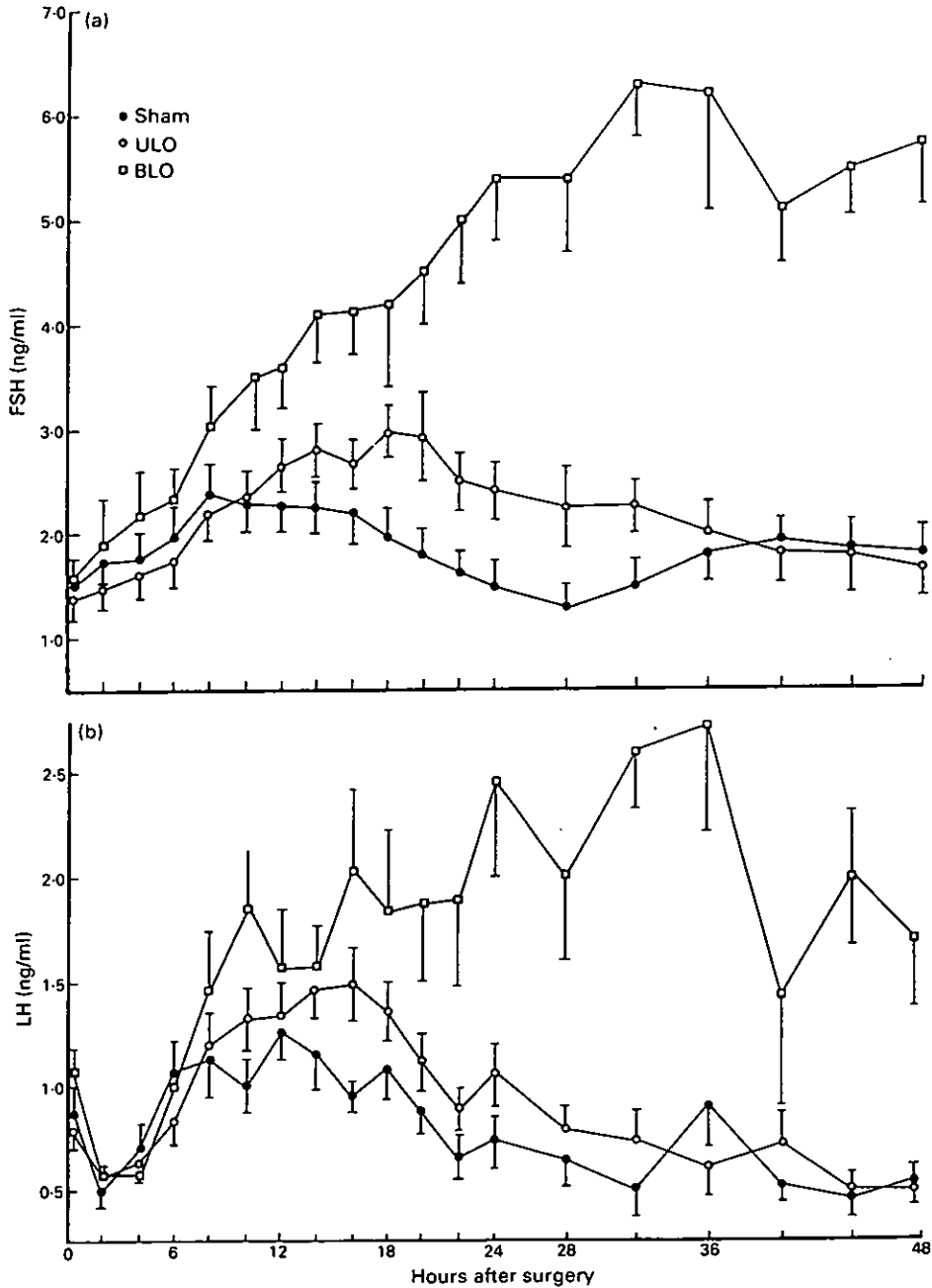
Fig. 6. Concentrations of (a) FSH and (b) LH after sham operation (Sham), unilateral (ULO) of bilateral (BLO) ovariectomy. (From Redmer *et al.*, 1984a.)

observed in cyclic gilts after a single injection of hCG in late proestrus (Eiler & Nalbandov, 1977) or after methallibure treatment (Hunter, Cook & Baker, 1976; Daguet, 1979; Gérard, Ménézo, Rombauts, Szöllösi & Thibault, 1979). Because of the similarity of preovulatory follicular steroidal development in gonadotrophin-stimulated prepubertal gilts and in cyclic gilts, it remains difficult to

explain the mechanism that suppresses first follicle development and the attainment of puberty in the prepubertal gilt.

Ovarian steroids feed back on the hypothalamus and pituitary to control LH secretion. Feedback control of FSH secretion is less understood. Although there is only one known releasing hormone for LH and FSH (Schally, Baba, Arimura, Redding & White, 1971), patterns of release of FSH and LH differ. To explain this difference, investigators have searched for a hypothalamic factor distinct from LHRH that releases FSH but not LH. Alternatively, the different patterns of LH and FSH release may be explained by an ovarian feedback mechanism selective for FSH. In prepubertal (65 days old), peripubertal, cyclic and pregnant gilts, unilateral ovariectomy results in compensatory growth of the other ovary as measured by increased number or growth of follicles and an increased volume of follicular fluid (Short, Peters, First & Casida, 1968; Dailey, Peters, First, Chapman & Casida, 1969; Dailey, Cloud, First, Chapman & Casida, 1970; Rexroad & Casida, 1976). In general, compensatory ovarian hypertrophy is characterized in the pig by an increased follicular growth rate and size and an increased volume of follicular fluid (Rexroad & Casida, 1976). There is discrepancy in the literature as to what physiological changes after unilateral ovariectomy cause hypertrophy of the remaining ovary. Peripheral hormone concentrations after removal of one ovary have not been well established in the pig. However, it has been suggested that, in the pig and other species (McLaren, 1963; Rexroad & Casida, 1976), decreased concentration of ovarian steroids, particularly oestradiol, and increased concentrations of FSH may be responsible for the resultant follicular growth (Short et al., 1968; Butcher, 1977). In the rat, however, Butcher (1977) and Welschen, Dullaart & de Jong (1978) observed that unilateral ovariectomy is not accompanied by decreased concentrations of peripheral oestradiol. In addition, steroid replacement does not inhibit compensatory ovarian hypertrophy except when pharmacological doses are given (Foote, Waldorf, Self & Casida, 1958; Ramirez & Sawyer, 1974; Butcher, 1977; Welschen et al., 1978). Butcher (1977) suggested that an ovarian factor other than oestrogen is reduced by unilateral ovariectomy, and this reduction produces a rise in FSH, resulting in follicular development and growth of the intact ovary in the rat.

Redmer, Christenson, Ford & Day (1984a) unilaterally ovariectomized prepubertal gilts at 130 days of age to investigate gonadotrophin secretion and compensatory ovarian hypertrophy. Unilaterally ovariectomized gilts released more FSH, but not more LH, during the first 24 h after surgery than did sham-operated gilts (Fig. 6). Release of FSH in unilaterally ovariectomized gilts was intermediate between that of sham-operated and bilaterally ovariectomized gilts. No differences were found in FSH and LH responses from 24 to 48 h after surgery for unilaterally ovariectomized and sham-operated gilts. These data indicate that unilateral ovariectomy causes a selective rise (12–24 h after operation) in FSH concentrations in the prepubertal gilt, as has been reported for rats (Butcher, 1977; Welschen et al., 1978; DePaolo, Anderson & Hirshfield, 1981), sheep (Findlay & Cumming, 1977; Smith, Agudo & Schanbacher, 1984), hamsters (Bast & Greenwald, 1977) and cattle (Johnson, Smith & Elmore, 1985).

After the increased FSH secretion on Day 1, Redmer et al. (1984a) showed an increase in ovarian venous oestradiol and an increase in follicular fluid weight on Day 2 but little change in wet ovarian weight by Day 2 for unilaterally ovariectomized gilts (Table 1). Thereafter, wet ovarian and follicular fluid weight increased through Days 4 and 8 after operation. After Day 4 ovarian venous oestradiol concentrations receded to values seen at Day 0 when the gilts were intact and similar to those of sham-operated gilts. Throughout the duration of this experiment, the peripheral concentrations of oestradiol were < 10 pg/ml for all gilts. Because ovarian weight increases after Day 4, one might expect ovarian venous oestradiol concentrations to increase rather than decrease. This would be expected if oestradiol was responsible for maintaining the inhibitory influence on the hypothalamic–pituitary control of gonadotrophin secretion. The decrease of ovarian venous oestradiol may be explained by at least two possible mechanisms. First, increased concentrations of a non-steroidal factor within the compensating ovary, with increased follicular growth, may antagonize FSH binding (Sato, Ishibashi & Iritani, 1982), and cause decreased oestradiol

Table 1. Ovarian venous serum oestradiol concentration, wet ovarian and follicular fluid weights in sham-operated and unilaterally ovariectomized (ULO) gilts (from Redmer *et al.*, 1984a)

Treatment	Day after operation		
	2	4	8
	Ovarian venous oestradiol conc. (pg/ml)		
Sham	460±126	416±149	273± 54
ULO†	258± 47	403±188	358± 54
ULO‡	1081±167*	1032±199*	334±167
	Wet ovarian weight (g)		
Sham	2·8±0·3	3·1±0·3	3·6±0·3
ULO†	3·0±0·2	3·8±0·6	3·6±0·3
ULO‡	3·4±0·4	5·1±0·4*	5·9±0·4*
	Follicular fluid weight (g)		
Sham	1·5±0·2	1·7±0·2	1·9±0·2
ULO†	1·6±0·2	2·0±0·3	1·9±0·2
ULO‡	2·0±0·2*	3·0±0·2*	3·4±0·2*

Values are mean ± s.e.m.
† Values for ovary removed at ULO on Day 0.
‡ Values for ovary removed 2, 4 or 8 days after ULO.
* $P < 0.05$ compared with value for sham-operated pigs.

production. Second, increased concentrations of another non-steroidal factor, due to increased follicular growth by the compensating ovary, may reduce peripheral FSH concentrations sufficiently to cause reduced ovarian production of oestradiol.

Is an inhibitory factor(s) removed at unilateral ovariectomy? What factor(s) is involved in the cessation of growth as compensation nears completion in the hypertrophic ovary? The first question was addressed by a determination of inhibin-like activity in the ovarian venous serum from unilaterally ovariectomized and sham-operated gilts (Redmer, Christenson, Ford, Day & Goodman, 1984b). Presumptive inhibin activity was always greater for ovarian venous samples than for peripheral serum samples (Table 2). For unilaterally ovariectomized gilts, inhibin activity increased 3-fold on Days 2 and 4 which corresponds to the time of elevated ovarian venous oestradiol concentration and presumably to the period of active follicular development in the unilaterally ovariectomized gilt. Although inhibin activity earlier than 2 days after operation was not evaluated, abrupt decreases in peripheral inhibin, as a result of removing one ovary, may allow FSH concentrations to rise sharply after unilateral ovariectomy.

The elevated ovarian venous inhibin activity detected on Day 2 may be derived from follicles recruited after the rise in serum FSH. Administration of follicular fluid during and after unilateral ovariectomy suppresses compensatory ovarian hypertrophy and subsequent follicular growth in mice (Sato & Ishibashi, 1977), pigs (Redmer *et al.*, 1985) and cows (Johnson *et al.*, 1985) and alters the profile of FSH release after unilateral ovariectomy (Redmer *et al.*, 1985). Increases in inhibin activity, alone or in combination with oestradiol, may therefore inhibit sustained increases of the rise in circulating FSH concentrations after unilateral ovariectomy. However, it is not clear what mechanism modulates the extent of follicular development and final ovarian size after unilateral ovariectomy. That is, compensatory ovarian hypertrophy begins with the rise in FSH but continues after FSH concentrations have returned to preoperative levels. Therefore, sustained elevations in

Table 2. Changes in inhibin activity after unilateral ovariectomy
(pFF equivalents, μg/ml) (from Redmer *et al.*, 1984b)

| Treatment	Day after operation		
	2	4	8
	Ovarian venous serum		
Sham	82 ± 29	73 ± 30	99 ± 48
ULO†	49 ± 12	55 ± 30	60 ± 30
ULO‡	$251 \pm 79*$	$275 \pm 111*$	68 ± 14
	Peripheral serum		
Sham	28 ± 17	37 ± 22	31 ± 17
ULO†	22 ± 10	18 ± 10	23 ± 15
ULO‡	50 ± 20	40 ± 14	28 ± 10

Values are mean ± s.e.m.
† Inhibin activity for ovarian venous or peripheral sample collected on
Day 0.
‡ Inhibin activity for ovarian venous or peripheral samples collected 2, 4 or
8 days after ULO.
* $P < 0.05$ compared with value for sham-operated animals.

circulating concentrations of FSH appear not to be the principal determinant of final ovarian follicular size.

Numerous non-steroidal regulators of follicular maturation have been suggested on the basis of studies with ovarian follicular fluid from pigs, cattle, and primates (Sato & Ishibashi, 1977; Sato, Miyamoto, Ishibashi & Iritani, 1978; Darga & Reichert, 1979; diZerega, Goebelsmann & Nakamura, 1982; Channing *et al.*, 1982; Fletcher, Dias, Sanzo & Reichert, 1982; Sluss & Reichert,
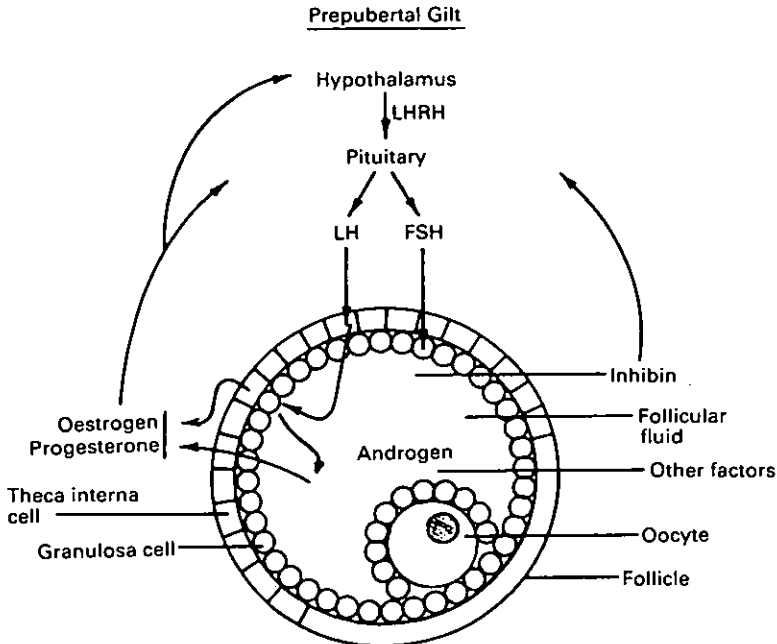
Fig. 7. Diagrammatic representation of a pig follicle and its control factors.

1984; Kling *et al.*, 1984). How these different regulators influence ovarian status of the prepubertal gilt is not understood.

From the reviewed information, the following follicle model is proposed for the prepubertal gilt (Fig. 7). By birth or during the early prepubertal period, the necessary oocytes and follicle populations are present and gonadotrophin-releasing hormone plus pituitary release of FSH and LH are established. From 60 to 100 days after birth, follicles become sensitive to gonadotrophins. As the number of granulosa cells increase in proliferating follicles, the number of FSH and oestrogen receptors increases. This proliferative process advances and FSH and small amounts of oestrogen in the tertiary follicles stimulate LH receptors on the theca interna cells which in turn secrete androgens and oestrogens. FSH and oestrogen-sensitive granulosa cells aromatize these androgens to oestrogens. Granulosa cells are responsible for follicular inhibin production which feeds back on the pituitary and possibly on the ovary. In pigs the role of non-steroidal regulators on ovarian function remains unknown. The factor(s) which prevents ovarian follicles in the prepubertal gilt from progressing to ovulation once they have acquired the ability to ovulate in response to exogenous gonadotrophins has still to be determined.

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