

Regulation and action of gonadotrophins in pigs

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Summary. Gonadotrophins, synthesized and secreted from the basophils of the adenohypophysis, bind to various target cells and elicit a wide variety of responses. Specific receptors for gonadotrophins have been found on plasma membranes of thecal, granulosa, luteal, endometrial and myometrial cells in the female and on Leydig and Sertoli cells in the male. Gonadotrophins exert their effects through various intracellular second messengers and control biosynthetic pathways of steroid production in responsive cells.

Gonadotrophins stimulate growth and development of antral follicles in the female. PMSG, FSH, or hourly pulses of GnRH, LH or a combination of LH and FSH induce follicular growth and development in prepubertal gilts and lactating and(or) anoestrous sows. The number of follicles that develop to ovulatory size in response to PMSG and FSH is dose-dependent, but pulsatile treatment with GnRH or gonadotrophins results in an ovulation rate similar to that observed during spontaneous follicular development. Endocrine changes resulting from treatments that induce follicular growth and development are similar to those observed during the follicular phase of the oestrous cycle.

Hypophysectomy, hypophysial-stalk transection, active and passive immunization against GnRH, and active immunization against LH impair reproduction by interfering with normal follicular development in the female. Gonadotrophins, administered to gilts as repeated injections of whole pituitary extract or as pulses of GnRH agonist, do not stimulate follicular growth in gilts actively immunized against GnRH. Similarly, PMSG is ineffective in inducing follicular growth and development in gilts actively immunized against GnRH and after hypophysectomy or hypophysial-stalk transection. In contrast, PMSG is effective for inducing follicular development in hypophysial stalk-transected pigs when pulses of GnRH are given simultaneously with the PMSG. These results suggest that agents in addition to the gonadotrophins are required for the full complement of follicular growth, recruitment and development. Insulin, growth factors and steroids modify the response of cells to the gonadotrophins and may mediate these effects. Other possibilities include substances released from the pituitary gland or GnRH-like peptide(s) produced by the ovary that act as autocrine or paracrine regulators of follicular development.

Gonadotrophins stimulate testicular function in the male. Active immunization of mature boars against GnRH or LH results in testicular atrophy, depressed steroidogenic and spermatogenic functions and impaired libido. Treatment of boars immunized against GnRH with hCG restores steroidogenic function of the testes as evidenced by testosterone production.

Gonadotrophins also exert an influence in the central nervous system. Administration of hCG intramuscularly or intracranially blocks the oestrogen-induced preovulatory LH surge in ovariectomized pigs via a short loop feedback control mechanism. Exogenous

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GnRH overcomes the hCG block, indicating that the site of gonadotrophin action is at the hypothalamus or higher brain centres. Although the short-loop feedback control of LH secretion is operative in mature gilts and newborn piglets, its exact role in the control of GnRH release from the hypothalamus remains unknown.

Introduction

Pig gonadotrophic hormones (luteinizing hormone, LH and follicle-stimulating hormone, FSH) are produced by the basophils, sometimes referred to as the gonadotrophs, within the adenohypophysis and exert their actions on various target tissues by triggering biochemical reactions. The gonadotrophic hormones, acting individually or synergistically, are responsible for follicular growth and development, ovulation and maintenance of the corpus luteum in the female and testicular steroidogenesis and spermatogenesis in the male. Gonadotrophins are produced in microgram quantities each day and their concentrations in peripheral circulation range from 10^{-9} to 10^{-11} mol/l. Gonadotrophins may elicit an immediate response, such as a pulse of LH causing almost instantaneous progesterone secretion from corpora lutea (Parvizi *et al.*, 1976) or oestradiol secretion from follicles of the prepubertal ovary (Flowers *et al.*, 1987), or a delayed response, such as ovulation in response to the surge in gonadotrophins (Hunter & Dziuk, 1968; Polge, 1969).

Pig LH and FSH are glycoproteins, each containing a hormone-non-specific α subunit and a hormone-specific β subunit. The primary structure of the non-specific α subunit is 90 amino acids (Maghuin-Rogister *et al.*, 1973), showing close homology with ovine and bovine α subunits (Strickland *et al.*, 1985). The ratio and sequence of amino acid residues in the β subunits of gonadotrophins are also highly conserved from species to species, with pig gonadotrophins having close homology with β subunits for gonadotrophins of other vertebrates (Sairam, 1983; Pierce & Parsons, 1981). Pig LH- β subunit has 119 amino acids (Maghuin-Rogister & Hennen, 1973) and pig FSH- β subunit has 107 amino acids (Closset *et al.*, 1978). Disulphide bridges are formed by cystine residues in the gonadotrophins; there are 5 disulphide bridges in the α subunit (Combarous & Hennen, 1974) and 6 in the β subunits (Sairam, 1983). The two subunits are held together by non-covalent bonds, forming the secondary and tertiary structure of the gonadotrophins (Closset *et al.*, 1978). Carbohydrates comprise between 15 and 20%, by weight, of the gonadotrophins. Constituent carbohydrate moieties are D-mannose, D-galactose, L-fucose, N-acetyl neuraminic acid, D-glucosamine and D-galactosamine. They contribute to the biological function of the gonadotrophins and to the differential rates of clearance from peripheral circulation (Pierce & Parsons, 1981).

Model of gonadotrophin action based on in-vitro studies

Specific receptors for pig LH have been found on granulosa (Channing & Kammerman, 1973; Channing, 1975; May *et al.*, 1980; May & Schomberg, 1981), thecal (Channing & Kammerman, 1974; Daguet, 1979; see Foxcroft & Hunter, 1985), luteal (Ziecik *et al.*, 1980; Ottobre *et al.*, 1984), endometrial (Ziecik *et al.*, 1986) and myometrial (Ziecik *et al.*, 1986; B. Flowers, A. J. Ziecik & E. V. Carvolo, unpublished) cells in the female. FSH specifically binds to granulosa cells (Channing & Kammerman, 1974; Nakano *et al.*, 1977, 1983), but does not bind specifically to luteal tissue during the oestrous cycle or pregnancy in pigs as it does in other species such as cattle and hamsters (Oxberry & Greenwald, 1982; Manns *et al.*, 1984; Ziecik *et al.*, 1988b). Gonadotrophin binding to cellular components within the ovary is responsible for stimulating growth of follicles that already have 4 layers of granulosa cells, stimulating formation of the antrum, ovulation and formation of corpora lutea, and maintenance of luteal function. The function of LH binding to the endometrium and myometrium is less apparent, but it may include involvement in gamete transport, maternal recognition of pregnancy, intrauterine migration of embryos and parturition.

In the male, LH specifically binds to the Leydig cells (Hall, 1970) and an LH agonist, human chorionic gonadotrophin (hCG), can stimulate testosterone production in early fetal, neonatal and mature pig testes (Raeside & Middleton, 1979; Peyrat *et al.*, 1981). Receptors for hCG/LH are found in neonatal pig testes (Ziecik *et al.*, 1987b) and increase in number until about 7 weeks of age. FSH specifically binds to Sertoli cells and, in conjunction with testosterone, controls the function and structure of this cell (Chevalier, 1979; Colenbrander *et al.*, 1982).

After binding to specific receptors, gonadotrophins elicit their biological response via various intracellular second messengers. The general consensus is that binding of the gonadotrophins to a receptor results in activation of adenylate cyclase, catalysing the conversion of ATP to cAMP (see Hunzicker-Dunn & Birnbaumer, 1985, for review). Accumulated cyclic AMP then interacts with protein kinases, specific intracellular proteins, releasing a catalytic subunit that induces the phosphorylation of other specific cellular proteins. These proteins are most often enzymes involved in steroidogenesis and lead to the functions for which the gonadotrophins are noted (Sairam, 1983; Hunzicker-Dunn & Birnbaumer, 1985). Gonadotrophins stimulate de-novo synthesis of steroids from acetate and conversion of cholesterol esters to steroids in steroid-secreting target cells.

Control of oestrogen biosynthesis by the ovary is a complex process involving interaction between granulosa and thecal cells of developing follicles. Studies in the rat have led to the two-cell hypothesis of follicular oestrogen production (Ryan, 1979; Hsueh *et al.*, 1984; Richards *et al.*, 1987). Evidence for a similar interaction in the pig and details of the regulation of ovarian endocrine function by the gonadotrophins are presented by Ainsworth *et al.* (1990).

Action of the gonadotrophins in gonadal function

Follicular development

Use of exogenous gonadotrophin. Administration of appropriate doses of exogenous gonadotrophins to anoestrous females results in follicular growth, ovulation and formation of corpora lutea. Casida (1935) was the first to report that precocious puberty could be induced in gilts with exogenous gonadotrophins. Since then numerous workers have reported the use of pregnant mare's serum gonadotrophin (PMSG) alone or in combination with hCG to stimulate follicular development in prepubertal gilts (reviewed by Paterson, 1982). Similarly, PMSG alone or in combination with hCG has been used to induce follicular development and ovulation in sows during lactation (Britt *et al.*, 1985) and in weaned, anoestrous sows (Schilling & Cerne, 1972; Dyck *et al.*, 1979; King *et al.*, 1982; Dial *et al.*, 1984).

Follicular growth has also been induced in the pig by treatment with pituitary preparations containing pig gonadotrophins. A crude FSH preparation induced follicular growth and ovulation sites when given to gilts during the follicular or luteal phase of the oestrous cycle (Spalding *et al.*, 1955; Day *et al.*, 1959). Treatment of lactating sows with 20 mg FSH for 4 or 5 days induced follicular growth with accompanying increases in follicular fluid weight and average diameter of follicles (Kirkpatrick *et al.*, 1965; Peters *et al.*, 1968, 1969). Superovulation can be induced in pigs by treatment with exogenous gonadotrophins (Webel & Day, 1982) and a positive correlation exists between the amount of PMSG or FSH given and the number of follicles which are stimulated to develop (Baker & Coggins 1966; Phillippo, 1968; Paterson, 1982).

Guthrie *et al.* (1988) have treated gilts with injections of 8 µg purified FSH (USDA-pFSH-B1) per kg of body weight every 8 h for 64 h, starting 48 h before the last feeding of altrenogest (a progestagen). Ovarian morphology was examined 24 h after withdrawal of altrenogest, which was 8 h after the last injection of FSH. Treatment with FSH elevated plasma concentrations of FSH and increased the number of follicles 3–6 mm in diameter, but did not influence plasma concentrations of LH, oestrogen or progesterone or influence the numbers of follicles 1–2 mm or 7–8 mm in diameter.

Gonadotrophin-releasing hormone (GnRH) induces the release of LH and FSH when given intravenously, subcutaneously or intramuscularly to pigs. Follicular development and fertile oestrus can be induced by pulsatile administration of GnRH in lactating sows (Britt *et al.*, 1985), weaned anoestrous sows (Armstrong & Britt, 1985) and prepubertal gilts (Lutz *et al.*, 1985; Carpenter & Anderson, 1985; Pressing *et al.*, 1987). Administration of 1.5–2.5 µg GnRH hourly or once every other hour elicits a predictable ovarian response, with gilts or sows exhibiting fertile oestrus between 90 and 120 h after initiation of treatment. The duration of oestrus, ovulation rate and endocrine changes in gilts and sows treated with pulses of GnRH are similar to those observed during spontaneous follicular development and oestrus.

Use of a pituitary extract. Since GnRH releases LH and FSH from the pituitary, we conducted several studies to determine whether pulsatile administration of a pig pituitary extract or purified LH and FSH would induce follicular development and oestrus in lactating sows and prepubertal gilts. A summary of this research is presented in Table 1. Lyophilized pig pituitary powder was suspended in saline, centrifuged, the supernatant decanted, twice passed through a 0.45 µm Millipore filter, and diluted to 3 µg LH and 0.4 µg FSH/ml with saline. The content of gonadotrophins in the pituitary extract was determined by radioimmunoassay, with LER 786-3 and USDA-pFSH-B1 used as the standards for the LH and FSH assays. Then 1 ml of the pituitary extract was given hourly for 120 h to sows and gilts via indwelling jugular cannula. Four of 4 lactating sows and 6 of 6 prepubertal gilts responded to this treatment by exhibiting oestrus 115 ± 5 (mean \pm s.e.m.) h after start of treatment. Each injection of pituitary extract resulted in a 0.78 ng/ml increase in immunoassayable LH within 5 min in sows and an increase of approximately 2 ng/ml in gilts, the difference in response being due to the difference in weight between these classes of animals (Table 2). Basal concentrations of LH, measured in samples taken every 6 h and defined as the mean value of all samples minus samples greater than two standard deviations above the mean, were similar in lactating sows and prepubertal gilts given saline. Basal concentrations of LH were elevated as a result of pulsatile treatment with a pituitary extract, whereas no change in circulating concentrations of FSH could be detected.

The sequence of endocrine events in sows and gilts responding to pulsatile treatment with a pituitary extract (Fig. 1) was similar to that observed in female pigs responding to pulsatile

Table 1. Summary of trials on induction of oestrus in lactating sows (S) or prepubertal gilts (G) by pulsatile administration of a pituitary extract or purified gonadotrophins

Treatment	Animal	Dose*		No. in oestrus (ovarian stimulation)/ no. treated	Mean \pm s.e.m. time (h) to oestrus
		LH	FSH		
Saline	S and G	0	0	3/17	100 \pm 2
Pituitary extract	S and G	3	0.4	10/10	115 \pm 8
Pig LH	S	0.58	0	1/4	96
		1.54	0	3/4	124 \pm 4
		2.89	0	0/4	—
Pig LH	G	0.75	0	1/4	84
		1.50	0	1/4	120
		3.00	0	1/4	120
		6.00	0	5/5	116 \pm 16
Pig LH + pig FSH	G	1.5	0.1	0/5	—
		1.5	0.2	0/5	—
		1.5	0.4	4/5	—

*Dose of LH and FSH in the pituitary extract was measured by radioimmunoassay procedures and represents the total amount given. Dose of purified LH (USDA-pLH-B1) and FSH (USDA-pFSH-B1) given were µg per 100 kg body weight. The pituitary extract and gonadotrophins were administered as discrete intravenous pulses every h.

Table 2. Hormone concentrations (ng/ml) in lactating sows and prepubertal gilts given saline or pituitary extract

Animal treated	Treatment	No. treated	Basal concentrations		Increase in peripheral circulation	
			LH	FSH	LH	FSH
Lactating sow	Saline	4	0.95 ± 0.01	22.4 ± 3.7	—	—
	Pituitary extract	4	1.40 ± 0.07*	8.7 ± 1.0	0.78 ± 0.09	0.6 ± 0.9
Prepubertal gilt	Saline	6	0.93 ± 0.05	19.7 ± 2.0	—	—
	Pituitary extract	6	1.22 ± 0.04*	14.4 ± 1.9	1.95 ± 0.14	4.5 ± 0.9

**P* < 0.05 compared to values for sows or gilts given saline.

treatment with GnRH and to that observed during the follicular phase of the oestrous cycle in females exhibiting spontaneous oestrus. Briefly, oestradiol remained low for the first 24–36 h of treatment and then gradually increased to a peak approximately 6–18 h before onset of oestrus, then declined precipitously. Gonadotrophins remained low until the preovulatory surge occurred.

Since all of the sows and gilts responded to pulsatile treatment with a pituitary extract by exhibiting follicular growth and secretion of oestradiol, it can be concluded that an increase in basal concentrations of LH or an intermittent delivery of the gonadotrophins to the ovary provided sufficient stimulus for sustained follicular growth and oestradiol production. It must also be recognized that hormones other than the gonadotrophins are synthesized in the adenohypophysis and therefore were given hourly to sows and gilts along with the gonadotrophins. The concentration of these hormones in the pituitary extract or their concentration in circulation after administration were not determined. The positive response observed in studies with gilts and sows given a pituitary extract may depend upon one of these other hormones or a synergistic involvement between one of these hormones and the gonadotrophins.

Use of purified gonadotrophins. In a subsequent series of experiments, purified pig LH (USDA-pLH-B1) was given to prepubertal gilts alone or in combinations with purified pig FSH (USDA-pFSH-B1). Intravenous administration of 0.75, 1.5 or 3.0 µg LH per 100 kg body weight hourly caused an increase in basal concentration of LH, but did not influence circulating concentrations of FSH or oestradiol or induce follicular development (Fig. 2). Treatment with 6 µg LH per 100 kg body weight induced oestrus in 5 of 5 prepubertal gilts. Each hourly injection of LH caused peripheral LH concentrations to increase an average of 3.19 ng/ml and mean basal LH concentration was 1.55 ng/ml (Fig. 3), both of which were higher than in saline-treated gilts and higher than in gilts responding to pulsatile treatment of pituitary extract. Follicular development and oestradiol production occurred in these gilts even though there was no detectable change in FSH concentrations.

Since more purified LH was required to induce follicular development and oestradiol production when given alone than when given as a component of the pituitary extract, an experiment was conducted to investigate the influence of giving both purified LH and purified FSH on follicular development. Prepubertal gilts were given 1.5 µg LH per 100 kg body weight, a dose known to increase peripheral LH values by about 0.8 ng/ml with each injection and not to cause follicular development. In addition, FSH was given at a rate of 0.1, 0.2 or 0.4 µg per 100 kg body weight. Treatments were given hourly for 120 h. As observed previously, administration of purified LH caused an increase in basal concentrations of LH. The higher hourly doses of FSH resulted in higher amounts of FSH and oestradiol in the circulation, even though an increase in immunoassayable FSH could not be detected in the circulation within 5 min after treatment. Of 5 gilts given 1.5 µg LH and 0.4 µg FSH per 100 kg body weight, 4 had sustained follicular growth and oestradiol production, although none of these gilts displayed oestrus in response to hand pressure to their backs. These gilts had induced corpora lutea or follicles > 10 mm in diameter when observed by

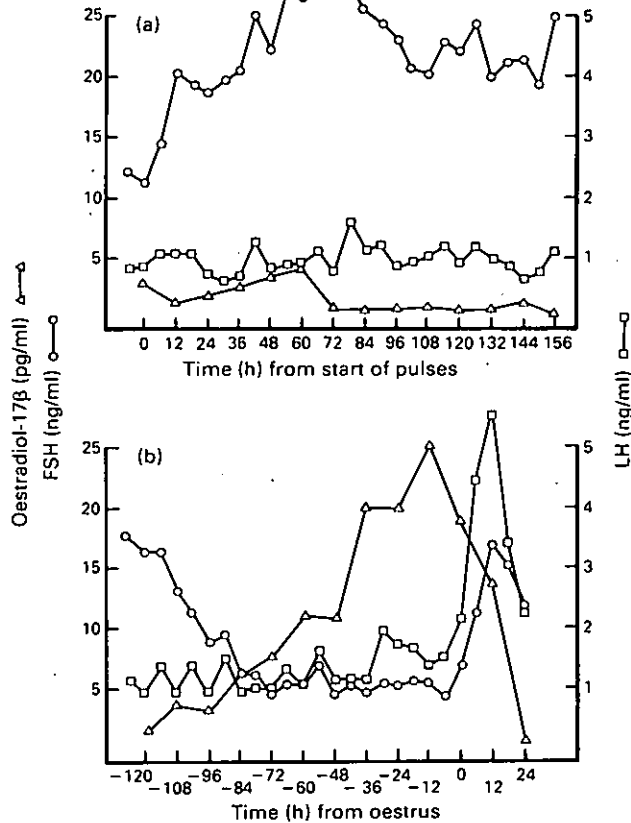


Fig. 1. Mean serum concentrations of LH, FSH and oestradiol-17 β in sows given pulses of saline (a) or pulses of a pituitary extract containing 3 μ g LH and 0.4 μ g FSH (b). The pituitary extract and saline were given as intravenous pulses every h. Values in (a) are plotted in relation to initiation of pulsatile treatments, while those in (b) are plotted in relation to the onset of oestrus. Standard errors ranged from 0.06 to 1.21 ng/ml for LH, 1.0 to 8.9 ng/ml for FSH and 0.1 to 4.8 pg/ml for oestradiol-17 β and were generally proportional to the mean.

laparoscopy at the conclusion of the treatment period. The sequence of hormonal changes that occurred in gilts that responded to pulsatile treatment with purified LH or a combination of LH and FSH by exhibiting oestrus or with follicular development was similar to that observed during spontaneous oestrus or after treatment with GnRH or pituitary extract.

There were no superovulatory effects from the treatment of sows or gilts with gonadotrophins. The numbers of corpora lutea and/or follicles obtained from stimulation by pituitary extract, high levels of purified LH or a combination of LH and FSH were similar to those of controls, to those reported for sows and gilts treated with pulses of GnRH, and to those expected for the herd from which the experimental animals came.

Gonadotrophin deprivation and replacement therapy. Gonadotrophin deprivation results in an impairment of reproduction. Hypophysectomy and hypophysial-stalk transection causes a reduction in pituitary gland weight, a depression in circulating concentrations of the gonadotrophins, a reduction in total ovarian and follicular fluid weight, and regression of Graafian follicles, with all follicles becoming atretic by 8–12 days after surgery (Anderson *et al.*, 1967; Kraeling *et al.*, 1974, 1986; Berardinelli & Anderson, 1981; Klindt *et al.*, 1983). Similarly, Esbenshade & Britt (1985) and Traywick & Esbenshade (1988) reported that active immunization of sexually mature gilts against

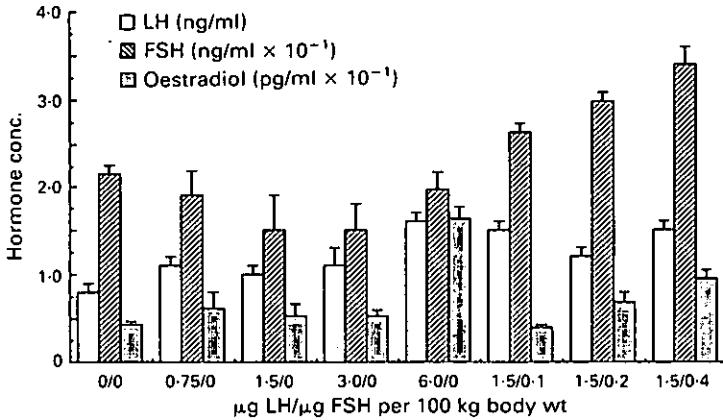


Fig. 2. Concentrations of LH, FSH and oestradiol in prepubertal gilts given (i.v.) various combinations of LH (USDA-pLH-B1) and FSH (USDA-pFSH-B1) hourly for 120 h. Values are the mean \pm s.e.m. of 4–6 observations for basal concentrations of the respective hormones.

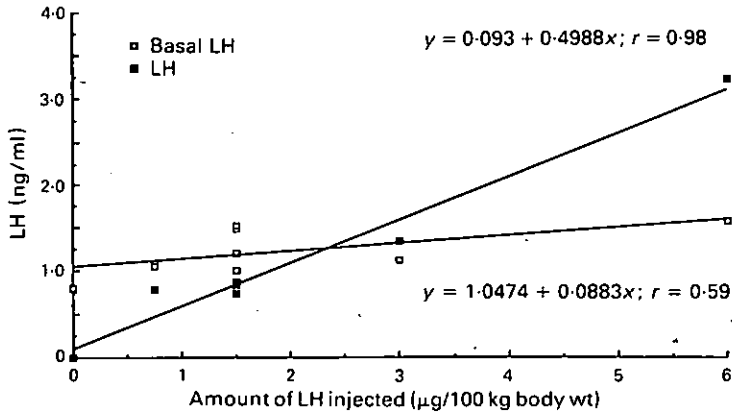


Fig. 3. Relationships between amount of pig LH given on a body weight basis and basal concentrations of LH and change in LH after an i.v. injection of purified LH. The purified LH (USDA-pLH-B1) was given hourly for 120 h. Basal concentrations were estimated as the sum of samples taken every 6 h minus values greater than two standard deviations from the mean and the increase in LH in response to injection was determined on the day after initiation of pulsatile treatment.

GnRH induces acyclicity, suppresses concentrations of gonadotrophins and oestradiol, and causes atrophy of the ovaries. Ovaries taken from gilts actively immunized against GnRH contained few antral follicles and more than 98% of follicles with more than 4 layers of granulosa cells were atretic, as manifested by the presence of pycnotic bodies (Esbenshade, 1987; Traywick & Esbenshade, 1988).

Replacement therapy with gonadotrophins has been used in gilts that have been hypophysectomized, hypophysial-stalk transected or actively immunized against GnRH in attempts to induce follicular growth and development. A single injection of 1000 i.u. PMSG 2 or 5 days after hypophysectomy, 2 days after hypophysial-stalk transection, or in gilts actively immunized against GnRH failed to stimulate follicular growth (Kraeling *et al.*, 1974, 1986; Esbenshade, 1987). Esbenshade (1987) gave gilts actively immunized against GnRH increasing doses of PMSG (from 200 to 1600 i.u.) every 3rd day for 48 days and did not detect follicular development. The failure of

PMSG to stimulate follicular growth in these animals was complete, as recovered ovaries showed no increase in follicular fluid weight and had only small antral follicles < 1 mm in diameter.

An aqueous extract of lyophilized pituitaries, prepared as described previously, given to hypophysectomized gilts or gilts actively immunized against GnRH has produced conflicting results. Kraeling (1970) reported that daily intramuscular injections of a pituitary extract induced follicular growth and ovulation, but Esbenshade (1987) gave a pituitary extract intravenously every 6 h for 9 days and did not observe a stimulation of follicular growth or an increase in peripheral concentration of oestradiol. Anderson *et al.* (1967) gave desiccated pig anterior pituitary to mated, hypophysial stalk-transected gilts for at least 20 days and this treatment maintained corpora lutea, progesterone production and pregnancy, but they reported that only atretic follicles were observed in the ovaries at the conclusion of treatment.

Traywick & Esbenshade (1988) gave 100 ng GnRH agonist at 2-h intervals for 72 or 144 h to gilts actively immunized against GnRH. This treatment did not induce follicular development, change pituitary or uterine weights, change the population of follicles in the ovaries or alter the incidence of

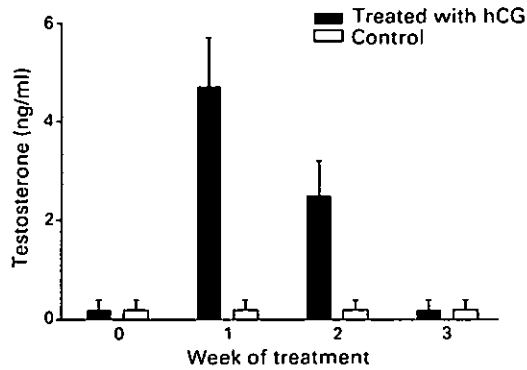


Fig. 4. Concentration of testosterone (mean \pm s.e.m.) in boars actively immunized against GnRH and given 500 i.u. hCG every other week or in boars actively immunized against GnRH and not treated. N = 5/group.

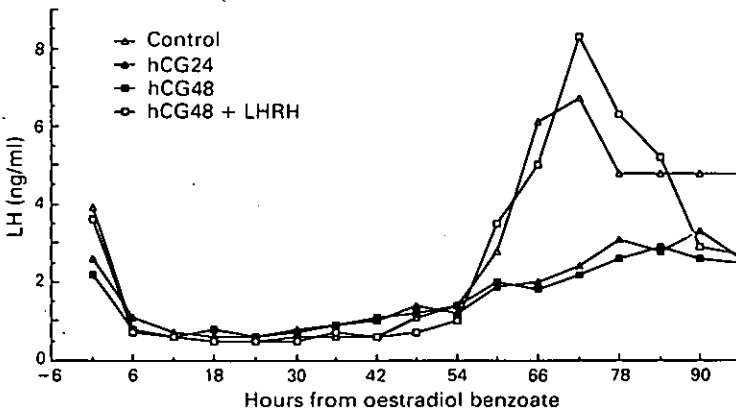


Fig. 5. Concentration of LH in ovariectomized gilts given oestradiol benzoate at time 0 and 2000 i.u. hCG 24 or 48 h later, or treated with 2000 i.u. hCG at 48 h after oestradiol and then given hourly pulses of 100 ng GnRH-agonist from 54 to 96 h after oestradiol. Values represent mean of 6 animals.

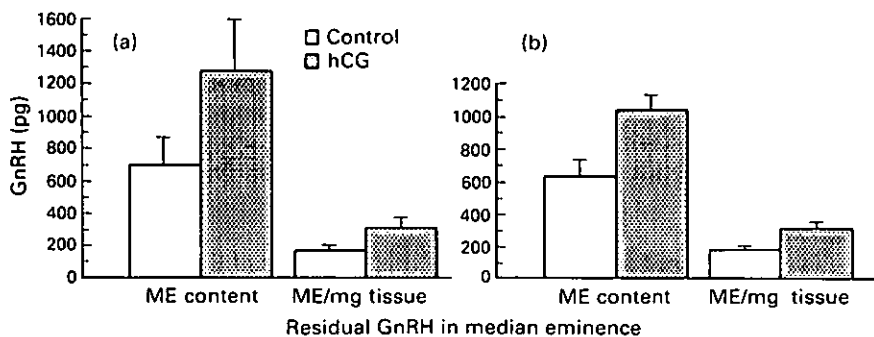


Fig. 6. Total content of GnRH and GnRH content per mg tissue in the median eminence (ME) in (a) male and (b) female piglets 14 days of age that were treated with saline (control) or hCG (100 i.u./kg body wt) 24 h before recovery. Tissue was incubated for 120 min *in vitro* and challenged twice with 56 mM-K⁺ before determination of residual content of GnRH. Values represent mean \pm s.e.m. for 6 observations.

atresia, but GnRH-immunized gilts pulsed with GnRH agonist for 72 h had significantly heavier ovaries than did those in the other treatment groups.

Kraeling *et al.* (1987) gave hypophysial stalk-transected pigs 2.5 μ g GnRH every 45 min for 9 days. Gilts were then given 1000 i.u. PMSG on Day 7 and ovaries were recovered on Day 10. This regimen increased ovarian and follicular fluid weight and induced follicular growth, with preovulatory follicles present at Day 10.

One explanation of these results is that hypophysectomy, hypophysial-stalk transection and GnRH immunization caused an irreversible loss of gonadotrophin receptors in the ovaries due to the non-detectable levels of gonadotrophins produced in these experimental animals. These animals therefore could not respond to treatment with exogenous gonadotrophin, but could respond if gonadotrophins in the circulation were maintained by treatment with GnRH. Evidence to refute this notion includes the induction of follicular growth in hypophysectomised gilts with a pituitary extract (Kraeling, 1970) and the observation that acyclic gilts immunized against GnRH eventually resume normal reproductive cycles and reproductive function (K. L. Esbenshade, unpublished data).

On the other hand, these results could suggest that agents in addition to the gonadotrophins are required for the full complement of follicular growth, recruitment and development. One such possibility would be some factor(s) released from the pituitary or released in response to other pituitary hormones that would be required to maintain the ovary in a state of readiness to respond to gonadotrophins. Various substances have been shown to mediate the effects of the gonadotrophins at the level of the ovary. For example, insulin (Hammond *et al.*, 1983), growth factors (Hammond *et al.*, 1988), glycosaminoglycans (Ax & Bellin, 1988) and steroids (Richards, 1979) can modulate gonadotrophin binding to cells of the ovary and, of the pituitary hormones, prolactin may have important effects on ovarian steroidogenic activity, as described by Dusza & Tilton (1990). It is possible that a substance synthesized and released by the pituitary in response to GnRH is required for maintenance of gonadotrophin receptors or interacts with the gonadotrophins at the level of the ovary for the gonadotrophins to exhibit their full functionality.

Another possibility is that GnRH may act directly at the level of the ovary to modulate function of the gonadotrophins. In the rat, specific high-affinity binding sites for GnRH are present on granulosa cells that have affinity properties indistinguishable from GnRH receptors in the pituitary gland (Jones *et al.*, 1980; Reeves *et al.*, 1980; Pieper *et al.*, 1981). Various functions have been attributed to the action of GnRH at the level of the ovary, including a stimulatory and inhibitory effect on steroidogenesis. Rivier & Vale (1989) have provided evidence that an endogenous GnRH-like peptide of ovarian origin interferes with FSH-induced inhibin release. Brown & Reeves (1983),

utilizing crude and membrane-enriched homogenates of ovarian tissue, reported a lack of specific GnRH receptors on pig ovaries, but Massicotte *et al.* (1980) observed a reduction in the FSH receptor content in pig granulosa cells when incubated with a GnRH agonist. The lack of response to gonadotrophins in animals after manipulation of gonadotrophin and GnRH concentrations via hypophysectomy, hypophysial-stalk transection and active immunization against GnRH may be due to GnRH or GnRH-like peptide acting as an autocrine or paracrine regulator of folliculogenesis.

Testicular function

Active immunization of the boar against GnRH results in a reduction in circulating concentrations of the gonadotrophins, gonadal atrophy and loss of libido (Falvo *et al.*, 1986; Grizzle *et al.*, 1987; Esbenshade & Johnson, 1987). Histological observation of testes from boars actively immunized against GnRH showed a reduction in seminiferous tubule diameters, disruption of Sertoli cells and a reduction in the size and cytoplasmic structures of Leydig cells (Grizzle *et al.*, 1987; Awoniyi *et al.*, 1988b). Falvo *et al.* (1986) attempted to actively immunize growing boars against ovine LH or porcine LH. Boars immunized against ovine LH did not respond to treatment, but boars immunized against porcine LH had lower testosterone concentrations and lighter accessory sex gland weight than did control boars.

Several attempts have been made to re-establish Leydig cell function in boars actively immunized against GnRH. Intravenous injection of 1 µg LH/10 kg body weight significantly increased plasma testosterone concentrations at 60 min in boars actively immunized against GnRH, but the magnitude of response was smaller than for control boars. Similarly, testosterone secretion in testicular fragments incubated *in vitro* with exogenous LH were significantly lower in GnRH-immunized boars than in control boars (Awoniyi *et al.*, 1988a). When 500 i.u. hCG were given i.m. to boars actively immunized against GnRH every other day for 3 weeks, production of testosterone was induced, with maximal concentrations observed 1 week after initiation of treatment (K. L. Esbenshade, S. K. Johnson & B. H. Johnson, unpublished data). Serum testosterone then returned to pre-treatment levels by the 3rd week of treatment even though injections of hCG were continued (Fig. 4). These results indicate that the Leydig cells deprived of gonadotrophins via immunoneutralization can respond to exogenous gonadotrophins, albeit to a lesser degree and for a limited period of time.

Action of the gonadotrophins in the central nervous system

LH may participate in the control of its own secretion through short-loop feedback at the level of the GnRH-secreting parvocellular neurone or at other neurones that impinge upon the GnRH secretory system. The concept of short-loop feedback originally was based on retrograde flow of blood within the portal system between the median eminence and the anterior pituitary of rats (Oliver *et al.*, 1977). There are now questions about whether retrograde flow plays an important role in autoregulatory feedback, but there is increasing evidence (Hostetter *et al.*, 1987) that gonadotrophins are found within the brain, especially in the medial basal hypothalamus, and that they may alter GnRH secretion and thereby affect secretion of pituitary gonadotrophins (Emmanuele *et al.*, 1981).

The first evidence that short-loop feedback might be important for regulating LH in the pig was from a study by Ziecik *et al.* (1987a), in which hCG was given to gilts previously treated with altrenogest and PMSG to synchronize oestrus. Altrenogest was fed for 14 days and 750 i.u. PMSG were given on the last day of feeding. Then, gilts (N = 6) were given 0, 500, 1000 or 1500 i.u. hCG, 72 h after the injection of PMSG. Of 12 gilts treated with the two higher doses of hCG, 5 did not have a surge of endogenous LH associated with the synchronized oestrus. It was not certain whether the absence of an LH surge in these gilts was due to decreased secretion of oestrogen and increased secretion of progesterone in response to the luteotrophic effects of hCG or to a direct short-loop effect of hCG at the hypothalamus or anterior pituitary.

A more comprehensive examination of short-loop feedback regulation of the LH surge was conducted by Ziecik *et al.* (1988a). Ovariectomized gilts were given oestradiol benzoate (10 µg/kg at 0 h) to induce a surge of LH between 54 and 96 h. To evaluate whether hCG would block the oestradiol-induced surge of LH, these gilts were given saline or hCG (2000 i.u.) at 24 or 48 h after the oestradiol benzoate. None of the gilts treated with hCG had an LH surge: however, during the period of expected positive feedback (54–96 h), LH returned to levels similar to concentrations noted before treatment with oestradiol (Fig. 5). These results showed that hCG did not modify the duration of negative feedback, but it always prevented the positive feedback surge of LH. To determine whether the action of hCG was at the anterior pituitary or higher centres, gilts were treated with oestradiol benzoate and hCG (2000 i.u. at 48 h) and then given hourly pulses of 100 ng of an LHRH-agonist from 54 to 96 h. Gilts treated in this manner all experienced a surge of LH at the expected time, indicating that hCG did not affect the pituitary's response to GnRH. The effects of hCG on FSH secretion were similar to those observed for LH, providing additional evidence that hCG exerted its effect by restraining secretion of GnRH.

Preliminary studies have been conducted to determine whether hCG acts at the 'GnRH pulse generator' and at the 'GnRH surge centre' or both. To evaluate the effects of hCG on pulsatile secretion of LH, ovariectomized gilts were sampled at various intervals from 24 h before until 96 h after intramuscular treatment with 500 or 2500 i.u. hCG (A. J. Ziecik, J. H. Britt & K. L. Esbenshade, unpublished observations). There was no evidence that hCG exerted chronic effects on episodic secretion of LH, but the sampling frequency was not frequent enough to rule out transient effects. In preliminary studies, hCG (100 or 1000 i.u.) was infused directly into lateral ventricles of the brain of 4 ovariectomized gilts (R. R. Kraeling & A. J. Ziecik, unpublished observations). In 4 of 6 instances, infusion of hCG apparently caused transient (2–4 h) cessation of episodic pulses of LH. In 2 additional gilts, hCG (1000 i.u.) was infused into a lateral ventricle 48 h after injection of oestradiol benzoate: the LH surge was blocked in 1 of these gilts.

In addition to studies with ovariectomized gilts, we (A. J. Ziecik, D. E. Morbeck, J. H. Britt & K. L. Esbenshade, unpublished) have conducted studies with intact neonatal male and female and castrated male piglets, because the neonatal pig secretes relatively high amounts of LH (Colenbrander *et al.*, 1987). Pigs were treated with hCG (100 i.u./kg) on Day 14 of age. In some piglets, blood samples for determination of steroids and gonadotrophins were collected for 48 h after treatment. In other piglets, the stalk median eminence and the medial basal hypothalamus were removed 24 h after hCG and incubated *in vitro* for 120 min to determine the rate of release of GnRH. In the initial hour of incubation, basal secretion was estimated by changing the medium every 30 min. Then the tissue was incubated for two 30-min periods in the presence of 56 mM-K⁺ to induce release of GnRH. Treatment with hCG reduced peripheral LH for more than 48 h in intact males and for 36 h in intact females. In males, hCG caused a transient (18 h) rise in testosterone, but it did not influence oestradiol in females. In males castrated at birth and treated on Day 14, hCG was without effect, but when males were castrated on Day 14 and treated immediately with hCG, LH was suppressed for 24 h. The basal and K⁺-induced release of GnRH from the median eminence were similar among males and females and were not influenced by pretreatment with hCG. However, the amount of residual GnRH in median eminence tissue after incubation was significantly greater in tissue from males and females treated with hCG than in tissue from their saline-treated contemporaries (Fig. 6). These results provide evidence that hCG may lower the proportion of GnRH-containing granules that are released from the median eminence terminals, and that this may be a steroid-dependent process. If the proportion of GnRH that is released is critical for assuring that the pituitary is exposed to a minimum threshold for induction of the LH surge (Kraeling & Barb, 1990), then hCG may keep the level below this threshold and thereby prevent large pulses or surges of LH.

The foregoing discussion has dealt with experiments that involved use of hCG as an LH agonist. It has not yet been shown in the pig that purified LH will act in exactly the same manner as hCG. Moreover, it is not known what level of endogenous LH might be necessary for short-loop feedback.

Nevertheless, the results of several experiments with pigs illustrate the potential role that LH or an LH agonist might play in regulating secretion of the gonadotrophins.

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