Postnatal ovarian follicle development in hypogonadal (*hpg*) and normal mice and associated changes in the hypothalamic–pituitary ovarian axis

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Summary. Significant uterine growth occurred in normal and hypogonadal (*hpg*) mice between Days 7 and 21 but thereafter no further growth was observed in *hpg* mice. The ovaries of *hpg* mice were significantly smaller than those of normals at all ages, but there was no significant difference between the number of non-growing follicles in the ovaries of mutants and their normal littermates at any age studied, and normal and *hpg* mice showed a marked reduction in the number of non-growing follicles during the first month of life. The size and composition of the growing follicle population in *hpg* mice, however, differed markedly from those in normal animals and by 21 days of age the number of growing follicles in mutants was significantly reduced. There was no significant difference in the number of Type 3b follicles before 60 days of age, but the number of all other follicle types was significantly less in *hpg* mice at all ages studied. Follicles in which the antrum is fully developed (Type 7 and 8) were never seen in the ovaries of mutants and corpora lutea were never observed. Interstitial tissue development was also very poor in *hpg* ovaries.

The hypothalamic GnRH content in normal mice remained low until Day 20, before rising sharply to adult levels (~800 pg) between Days 20 and 30. The pituitary FSH content increased over the first 10 days of life to reach a peak of about 5000 ng, before declining to the adult value of about 2000 ng by Day 30, whilst the plasma FSH concentration was high in the first 10 days, but fell to adult levels over the next 20 days. Pituitary LH content increased significantly between Days 5 and 10 to reach the adult level of about 600 ng.

Hypothalamic GnRH was undetectable at all ages in hypogonadal mice, but the pituitary content of FSH and LH had risen to the attenuated mutant adult value by Day 15, and unlike normals, plasma FSH concentrations were not elevated during the neonatal period.

These results suggest that minimal gonadotrophic stimulation of the ovary from birth has no effect on the total number of follicles but reduces the number of growing follicles and prevents follicle growth beyond the early antral stage. Gonadotrophins therefore appear to have a role in the initiation and continuance of follicle growth in the adult mouse.

Introduction

The development of the follicle population in mice has been studied in a variety of strains (Jones & Krohn, 1961a; Peters, 1969), and in animals with mutations affecting reproductive function (Howe,

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Lintern-Moore, Moore & Hawkins, 1978). Hypogonadal (*hpg*) mice are deficient in hypothalamic GnRH (Cattanach, Iddon, Charlton, Chiappa & Fink, 1977). The GnRH gene in *hpg* mice is grossly aberrant, lacking 2 exons (P. Seeburg, personal communication). Adult animals have an associated reduction in pituitary FSH and LH content and plasma FSH concentrations, and severe hypogonadism. The pituitary gland of *hpg* males and females is able to respond to injections of GnRH (Charlton, Fink & Halpin, 1981; Charlton *et al.*, 1983) and to GnRH released from normal hypothalami transplanted into the third ventricle of mutants (Krieger *et al.*, 1982). When transplanted into normal animals, ovaries from *hpg* females are capable of producing preovulatory follicles and releasing ova that can be fertilized (Bamber, Iddon, Charlton & Ward, 1980), and are therefore capable of normal function given the correct hormonal stimulus. The *hpg* mouse therefore provides an excellent model for studying the importance of gonadotrophins for the development of normal ovarian function. Here, we describe the post-natal histological development of the ovary, the uterus and the ovarian follicle population in *hpg* mice in comparison with normal litter mates. The changes in hypothalamic GnRH content, pituitary FSH and LH content and plasma FSH concentrations over the same period in both normal and *hpg* mice are also presented.

Materials and Methods

The mice used in these studies were *hpg* mutants or normal animals reared in the Department of Human Anatomy, Oxford, from an original stock provided by the MRC Radiobiology Unit, Harwell. All animals were a house strain derived originally from F₁ hybrids of two inbred strains, C3H/HeH and 101/H (Cattanach *et al.*, 1977). Females were weaned at 18–20 days of age and housed in groups of 4–6 in plastic cages (13 × 30 × 16.5 cm). All animals were maintained under controlled lighting conditions of 14 h light:10 h darkness (lights on 07:00 to 21:00 h) with free access to diet FFGM (Dixon & Sons Ltd, Ware, U.K.) and tap water.

The day of birth was designated as Day 0. Animals younger than 20 days were identified retrospectively on the basis of pituitary gonadotrophin and hypothalamic GnRH radioimmunoassay and *hpg* mice were distinguished from normals at laparotomy from 20 days of age. From 30 days of age *hpg* females could be distinguished from normals by the failure of vaginal opening and were housed separately.

Groups of virgin *hpg* and normal mice were killed at various ages, from 7 to 365 days and, in line with other studies (e.g. Jones & Krohn, 1961b), it was considered more important to kill normal animals at an exact age than on a specific day of the oestrous cycle in view of the great irregularities in the timing of the mouse cycle. Animals were anaesthetized with urethane (10 g ethyl carbamate/kg body wt, administered intraperitoneally as a 10% solution in 0.9% (w/v) NaCl), and the abdomen was opened. Ovarian blood vessels were clamped and the ovaries were removed and dissected free of surrounding fat. One ovary was fixed in 2% glutaraldehyde solution for a future separate ultrastructural study, and the other in Bouin's fluid for light microscopy. The uterus was also removed and dissected free of fat, fixed in Bouin's fluid and weighed after fixation.

Separate groups of animals were used for the study of hormone concentrations. Animals were anaesthetized with urethane as described above, and blood from the external jugular vein was sampled into heparinized syringes. It was centrifuged immediately and the plasma was separated and stored at –20°C. After sampling, the mice were killed by decapitation and the pituitary gland was removed and homogenized in 1 ml 0.9% phosphate-buffered saline (pH 7.2) and stored at –20°C. A block of tissue extending from the caudal edge of the optic chiasma to immediately caudal of the mammillary bodies, as wide as the median eminence and about 4 mm deep was removed and homogenized in 1 ml 0.1 M-HCl and stored at –20°C.

The ovaries for light microscopy were embedded in paraffin wax and sectioned serially at 7 µm. The sections were stained with haematoxylin and eosin. For each animal the total number of follicles of each type was determined from these sections, using the scheme of Pedersen & Peters

(1968). This is based on the size of the oocyte, the number of granulosa cells and the number of granulosa cell layers in the largest cross section of the follicle. Type 1 follicles have no cells attached to the oocyte and Type 2 have a few cells but not a complete ring. Type 3a follicles have a complete ring but this consists of fewer than 20 cells on the largest cross-section, whereas those of Type 3b have between 20 and 60. Type 4 follicles have two layers of granulosa cells with 60–100 cells. Type 5a is a transitional stage in which the oocyte has reached its maximum diameter in some follicles and there are three layers of cells (numbering 100–200). Follicles of Type 5b have a fully grown oocyte surrounded by many layers of cells but no follicular fluid is present, whereas Type 6 follicles again have many layers of cells but these are separated by scattered areas of fluid. Type 7 follicles have a single cavity containing follicular fluid but there are not more than 600 cells on the largest cross-section and although the cumulus oophorus has formed there is no stalk. Follicles of Type 8 also have a single cavity but the stalk is now well developed and these represent preovulatory follicles.

Growing follicles (i.e. Type 3b and larger) in every third section were counted and classified, and preliminary studies showed that this counting method enabled every follicle of Type 3b or larger to be identified and counted individually, and thus no correction factor was used. Non-growing follicles (Type 3a and smaller) were counted in every 10th section.

Hypothalamic GnRH content was determined by the method of Nett, Akbar, Niswender, Hedlund & White (1973) as used in this laboratory by Chiappa & Fink (1977) and Cattanach *et al.* (1977). Synthetic GnRH (ICI Ltd, Pharmaceuticals Division, Macclesfield, U.K.) was used as standard. The lower limit of sensitivity was 3 pg/tube and the inter- and intra-assay coefficients of variation were 11% and 13% respectively.

Pituitary LH was measured by the radioimmunoassay of Niswender, Midgley, Monroe & Reichert (1968) as used in this laboratory (Chiappa & Fink, 1977; Cattanach *et al.*, 1977). The standard was NIH-LH-S18, and the homogenates of pituitary tissue were assayed in duplicates of 10 and 100 μ l for which the lower limits of sensitivity were 4 μ g and 0.4 μ g LH/l respectively. Plasma and pituitary FSH were measured with a NIADDK rat FSH kit according to the method of Daane & Parlow (1971) as used in this laboratory (Chiappa & Fink, 1977; Cattanach *et al.*, 1977) using NIADDK rat FSH-RP1 as standard. The plasma and pituitary samples were assayed in duplicates of 100 μ l for which the lower limit of sensitivity was 250 μ g FSH/l. The inter- and intra-assay coefficients of variation were 7.2% and 8.8% respectively for the LH assay and 12% and 8.3% for the FSH assay.

The statistical significance of differences between groups was assessed by using an analysis of variance.

Results

The hypogonadal (*hpg*) mutation has marked effects on ovarian follicular and accessory sexual tissue development. The uterine weights in *hpg* mice increased significantly between Days 7 and 21 ($P < 0.01$), but then remained essentially unchanged up to 365 days (Table 1). In contrast, in normal mice uterine weight increased steadily to reach a mean value at 365 days which was 50-fold greater than that in *hpg* mice of the same age (Table 1). Histologically, the uteri of mutant animals showed a reduced endometrial cell height, a lack of endometrial infoldings and development of the smooth muscle layers. The ovaries of the mutants were considerably smaller than those of normals with an average weight of 0.4 ± 0.2 mg compared with 5.3 ± 0.7 mg at 90 days of age. Corpora lutea were present in 50% of the normal animals at Day 38 and in all the normal animals by Day 60. Corpora lutea were never found in the ovaries of *hpg* females. Vaginal opening occurred between 25 and 30 days in normal females, and was never seen in *hpg* females.

There was no significant difference between the number of non-growing follicles in normal and *hpg* mice at any age (Table 2); however, in the normal and mutant animals there was a significant

Table 1. Uterine weight (mean $\pm$ s.e.m.) in normal and *hpg* mice at different ages (5–10 at each age)

Age (days)	Uterine weight (mg)	
	Normal	<i>hpg</i>
7	1.0 $\pm$ 0.01	0.9 $\pm$ 0.02
21	8.1 $\pm$ 1.3	6.4 $\pm$ 0.7
29	14.6 $\pm$ 1.0	4.7 $\pm$ 0.7
38	12.4 $\pm$ 2.5	3.6 $\pm$ 0.7
60	81.3 $\pm$ 12.2	6.0 $\pm$ 0.3
90	147.8 $\pm$ 21.7	4.2 $\pm$ 0.6
100	112.8 $\pm$ 13.5	4.5 $\pm$ 0.4
365	280.8 $\pm$ 32.5	5.6 $\pm$ 1.0

reduction in the number of growing follicles present within the ovary from birth to 29 days of age ($P < 0.01$). There was no significant difference in the number of growing follicles in normal or *hpg* females at 7 days of age; however, by 21 days there were significantly more growing follicles in normal animals ($P < 0.001$) and this difference was maintained at all other ages studied.

There were major differences in the size and composition of the growing follicle population between *hpg* and normal mice at all ages (Table 2). At Day 7 the numbers of Type 3b follicles were not significantly different in *hpg* compared with normal mice (Table 2); however, the *hpg* ovaries contained significantly fewer ($P < 0.05$) follicles of Types 4 and 5a (Table 2). No follicles larger than Type 5a are present in *hpg* or normal mice at this age. By 21 days the ovaries of normal animals contained follicles of Types 6 and 7 but these were absent in the ovaries of *hpg* mice, which also had significantly fewer ($P < 0.01$) follicles of all types, except Type 3b. By Day 29, pre-ovulatory, Type 8, follicles were present in normal but not *hpg* mice, and the numbers of follicles of all types, except 3b, were again significantly reduced ($P < 0.01$) in *hpg* compared with normal animals. By Day 60, the number of Type 3b follicles was also significantly reduced in *hpg* mice compared with normals ($P < 0.01$). These differences were maintained in all animals up to 120 days of age. Follicles of Types 7 and 8 were never seen in the ovaries of mutants and only occasionally were follicles of Type 6 present.

In addition to the decrease in Type 3b follicles in *hpg* mice there was also a significant ($P < 0.001$) although transient increase in the number of Type 5a and 5b follicles at Day 60. At all stages, however, the follicle population in *hpg* females differed from that seen in normal mice of the same or any other age.

The follicles present in the *hpg* mouse ovaries were the same size as those with the same structure in normal animals, and there was no difference in the size of oocytes within non-atretic follicles in normal or *hpg* mice. The ovaries of *hpg* mice did, however, contain a larger number of contracted follicles in which oocyte enlargement and lipid accumulation by the granulosa cells preceded lysis of the oocyte and degeneration of the granulosa cells, leaving the zona pellucida as the last recognizable remnant of the follicle (Peters, 1969). In addition, many large follicles showed signs of atresia. Interstitial tissue was present in the ovaries of mutant females but its extent was considerably less than that in normal ovaries.

The hypothalamic GnRH content in normal mice increased between Days 5 and 10 and showed a slight fall during the next 10 days, before rising sharply to adult levels of around 1000 pg/hypothalamus between Days 20 and 30 (Fig. 1a). The pituitary FSH content increased over the first 10 days of life to reach a peak of about 5000 ng/pituitary gland before declining to the adult value of ~ 2000 ng/pituitary gland by Day 30 (Fig. 1b), and it then remained constant at all other ages studied. Plasma FSH concentrations, on the other hand, were high in the first 10 days of life,

Table 2. The types of growing follicles in normal (N) and *hpg* mice at different ages

Age (days)	Group	No. of mice	Total no. of follicles		Follicle type							
			Non-growing	Growing	3b	4	5a	5b	6	7	8	
7	N	6	4690 ± 359	123 ± 10	59.6 ± 4.1	46.0 ± 4.8	17.6 ± 3.2	0	0	0	0	
	hpg	4	4400 ± 377	96 ± 14	73.8 ± 7.5	20.8 ± 8.3	1.8 ± 1.0	0	0	0	0	
21	N	6	3610 ± 245	321 ± 12	54.8 ± 3.1	142.3 ± 8.9	80.5 ± 4.4	31.0 ± 3.9	10.5 ± 2.1	2.0 ± 0	0	
	hpg	5	4010 ± 121	114 ± 12	87.6 ± 13.5	20.0 ± 1.3	4.8 ± 0.5	1.8 ± 0.4	0	0	0	
29	N	6	2090 ± 337	181 ± 17	34.7 ± 4.7	89.2 ± 7.7	33.8 ± 8.1	15.5 ± 3.1	3.0 ± 0.5	2.2 ± 1.2	3.2 ± 1.1	
	hpg	5	3490 ± 610	47 ± 7	24.0 ± 2.3	13.5 ± 3.6	6.0 ± 1.4	3.8 ± 1.1	0	0	0	
38	N	6	2500 ± 276	211 ± 14	46.8 ± 6.3	80.3 ± 11.0	48.8 ± 4.6	25.2 ± 2.5	9.2 ± 1.5	0.2 ± 0.2	0.3 ± 0.2	
	hpg	5	2790 ± 364	54 ± 7	29.2 ± 3.4	17.8 ± 1.8	3.8 ± 1.5	2.4 ± 1.0	1.2 ± 0.2	0	0	
60	N	10	2030 ± 198	201 ± 11	38.8 ± 3.4	78.9 ± 5.8	35.0 ± 4.5	30.5 ± 2.8	9.6 ± 1.6	4.5 ± 0.7	3.4 ± 0.6	
	hpg	10	2680 ± 173	63 ± 10	18.6 ± 2.7	20.2 ± 5.0	13.5 ± 2.8	10.2 ± 2.2	0.5 ± 0.4	0	0	
90	N	5	2220 ± 136	194 ± 13	48.8 ± 4.4	65.4 ± 5.6	41.8 ± 2.8	23.8 ± 3.2	9.0 ± 1.8	3.2 ± 0.7	2.2 ± 1.0	
	hpg	6	1640 ± 207	24 ± 4	8.2 ± 1.6	11.3 ± 2.4	2.8 ± 0.8	1.5 ± 0.2	0.3 ± 0.2	0	0	
120	N	6	1890 ± 276	267 ± 31	65.8 ± 8.0	104.0 ± 15.0	52.7 ± 9.9	24.8 ± 3.7	9.8 ± 2.0	5.0 ± 1.1	5.5 ± 0.8	
	hpg	6	2460 ± 318	33 ± 7	15.2 ± 3.0	11.0 ± 3.3	4.2 ± 1.5	2.3 ± 0.7	0.2 ± 0.2	0	0	
365	N	5	1080 ± 169	80 ± 14	29.0 ± 6.6	26.8 ± 4.7	10.6 ± 1.7	4.6 ± 1.6	4.8 ± 1.0	3.2 ± 1.0	0.8 ± 0.4	
	hpg	5	1250 ± 216	15 ± 3	10.2 ± 2.1	2.6 ± 0.8	0.8 ± 0.4	1.8 ± 0.9	0	0	0	

Values are mean ± s.e.m. for one ovary.

Fig. 1. Hormonal values in normal mice (open bars) and hypogonadal mice (solid bars) at different ages. In (a) hypothalamic GnRH was undetectable at all ages in *hpg* mice. In (c) plasma FSH was undetectable at all ages in *hpg* mice. The 40-day GnRH samples were lost due to a freezer malfunction. Values are mean $\pm$ s.e.m. for 10 mice at each age. *Below level of sensitivity of assay (< 420 ng/pituitary).

~ 1000 ng/ml, but fell over the next 20 days (Fig. 1c) to reach values which in most cases were undetectable (i.e. 300 ng/ml). The pituitary LH content increased significantly between Days 5 and 10, and then values remained at approximately the adult values (Fig. 1d).

Hypothalamic GnRH was undetectable at all ages in hypogonadal mice. The pituitary FSH content was below the level of detectability of the assay in *hpg* mice until Day 15, at which time the content was ~ 400 ng/pituitary gland (Fig. 1b), and it remained at about this value at all other ages studied. Plasma FSH concentrations were undetectable in *hpg* mice at all ages studied. LH was detectable in the pituitary glands of *hpg* mice on Day 5 and Day 10 and had increased by Day 15 to a value not significantly different from that measured in adult mutant animals (Fig. 1d).

Discussion

The hypogonadal mutation has marked effects on the synthesis and secretion of pituitary gonadotrophins and on gonadal and accessory sexual tissue development. In adult mutant females the pituitary contents of immunoreactive FSH and immunoreactive and bioactive LH are dramatically reduced compared with those in normal mice, and plasma FSH concentration levels are undetectable (Cattanach *et al.*, 1977; Fink, Sheward & Plant, 1984). The gonadotrophs in male *hpg* mice are less numerous and smaller than those in normal animals (McDowell, Morris & Charlton, 1982) but nevertheless do possess GnRH receptors, albeit at reduced concentrations (Young, Speight,

Charlton & Clayton, 1983). The gonads of both sexes possess receptors for FSH and LH (Charlton, Parry, Halpin & Webb, 1982) and can respond to gonadotrophins when transplanted into normal animals (Bamber *et al.*, 1980) or when the animal is treated with GnRH (Charlton *et al.*, 1983).

The changes in pituitary and plasma hormone levels with age in normal animals confirm earlier studies in the mouse (e.g. Dullaart, Kent & Ryle, 1975) and demonstrate that the same general pattern is present in our strain of mice. The prepubertal fall in FSH concentrations in both the pituitary and the plasma probably reflects the development of negative feedback, which may occur both because of increased steroid production and a fall in plasma binding-protein levels (Puig-Duran, Greenstein & MacKinnon, 1979). The pituitary LH content, on the other hand, did not appear to be sensitive to the development of negative feedback over this period, remaining constant at about the adult level from Day 10. This suggests that there is a differential effect of feedback on pituitary gonadotrophin content at this time, although the underlying mechanism remains unclear. Further, the initial rise in the pituitary content of both FSH and LH occurred before the hypothalamic GnRH content had reached its maximum value, but as there are no data available on the release of GnRH, any interplay between synthesis and release in the control of gonadotrophin synthesis cannot be inferred.

The data on the amounts of gonadotrophins in young hypogonadal mice show that there is an increase in the pituitary content of both LH and FSH at around Day 15, despite the fact that hypothalamic GnRH was undetectable at all ages. This may be the result of a change in gonadotroph activity, either inherent or secondary to pubertal changes in other endocrine axes, or cell division may still be occurring at this time and thus the increased content may reflect an increase in the number of gonadotrophs. In marked contrast to normal mice, plasma FSH concentrations were undetectable at all ages in hypogonadal mice, and this strongly suggests that hypothalamic GnRH is responsible for the secretion of FSH observed in the neonatal period in normal mice.

The present study shows that in spite of the marked deficiency of hypothalamic GnRH and pituitary and plasma gonadotrophins, differentiation of the ovary and folliculogenesis appear to occur normally in *hpg* female mice. The fact that there was no significant difference in the number of non-growing follicles between normal and *hpg* animals suggests that there is not an absolute requirement for GnRH-induced gonadotrophin secretion in the formation or maintenance of primary ovarian follicles.

In the adult ovary, the most obvious effect of the *hpg* mutation is the paucity of antral follicle development, and this is consistent with observations on the effects of the absence of gonadotrophins in hypophysectomized animals (e.g. Jones & Krohn, 1961b). There is, however, a significant difference between the effects of hypophysectomy and the *hpg* mutation: namely the lack of effect of the mutation on the rate of loss of non-growing follicles. This suggests that the effect of hypophysectomy on this process may be due to the withdrawal of hormones other than the gonadotrophins, such as growth hormone or thyroxine, congenital absence of which has been shown to affect ovarian function (Howe *et al.*, 1978; Bray & York, 1979; Beamer, Eicher, Maltais & Southard, 1981).

In normal mice, plasma FSH concentrations were high in the early postnatal period, and these may be important in regulating follicle growth. There are, however, conflicting views as to the action of gonadotrophins in early follicle development in rodents. It is generally accepted that both ovarian morphology and endocrine function are independent of gonadotrophins during the first week of life (Ryle, 1970; Eshkol & Lunenfeld, 1972; Goldenberg, Reiter & Ross, 1973; Schwartz, 1974; Ojeda, Andrews, Advis & Smith-White, 1980), but there is some evidence that gonadotrophins do affect the ovary during this period (Anderson, Schwartz, Nequin & Ely, 1976; Purandare, Munshi & Rao, 1976; Arendsen de Wolff-Exalto, 1982). The present results for *hpg* mice suggest that in the 7-day-old mouse follicle development up to stage 3b/4 proceeds normally in the absence of significant GnRH, and gonadotrophins. However, although follicles do develop beyond this stage their number is reduced and thus gonadotrophins do appear to regulate follicle development in the mouse at this age.

The fact that there were no significant differences in the number of Type 3b and 4 follicles in normal and *hpg* mice at Day 7, despite the high plasma concentrations of FSH observed in normal mice, may be explained by the fact that small growing follicles at this time may be unresponsive to the high levels of FSH, perhaps because of low numbers of FSH receptors on small follicles, although in the adult ovary follicles up to the antral stage appear to have approximately similar levels of FSH receptors (Richards, 1980). There may therefore be uncoupling of the receptor from second messenger formation as has been observed for LH at this time (Kolena, 1976; George, Catt & Wilson, 1979; Ojeda *et al.*, 1980).

The total number of growing follicles was also reduced in *hpg* females, and in normal mice a major determinant of this number is the size of the non-growing pool (Peters, 1979). The present results show that this is broadly the case in *hpg* mice. However, the number of growing follicles was reduced in *hpg* mice compared to normal females despite the fact that the size of the non-growing pool was not significantly smaller in mutant animals. The relationship between the number of non-growing and growing follicles may therefore be influenced by low concentrations of gonadotrophins modulating the initiation of follicle growth.

In contrast with the present data on *hpg* mice, Peters, Byskov, Lintern-Moore, Faber & Anderson (1973) have argued that gonadotrophin concentrations do not affect the number of small growing follicles in normal mice. However, Lintern-Moore (1977) later showed that PMSG could increase the number of growing follicles if given from the day of birth up to Day 5, and that this increase was still apparent at Day 10. On the basis of this she suggested that there may be a critical period between Days 0 and 5 when gonadotrophins can regulate the initiation of follicle growth, and this may explain the effect on the follicle population if there is a perinatal gonadotrophin deficiency in hypogonadal mice.

In the pubertal and mature animal, however, the number of follicles at all stages of growth, including 3b, was reduced by the mutation suggesting a dependence upon GnRH-induced gonadotrophin secretion. Although the number of growing follicles was reduced in *hpg* mice, those follicles that did grow appeared normal up to the pre-antral stage, and the rate of follicle growth appeared approximately the same as in normal mice.

The development of the follicle antrum is thought to be an FSH dependent process (Richards, 1980) and this is supported by the finding that true antral follicles were never observed in the ovaries of *hpg* mice. This observation, together with the lack of uterine development, provides further evidence that antral follicles are the major follicular source of ovarian steroids, since although reduced in number, medium sized follicles were not completely absent in *hpg* mice.

The ovaries of *hpg* and normal mice contained reduced numbers of non-growing and growing follicles at 365 days. This suggests that in both mutant and normal ovaries follicular exhaustion occurs (Peters & McNatty, 1980), and that gonadotrophins are not essential for this process. However, the reduction was more severe in normal females, reinforcing the idea that low circulating gonadotrophin concentrations lead to a reduction in the rate of loss of follicles from the non-growing pool.

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