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Research

Age-related uterine and ovarian hypertrophy in FSH receptor knockout and FSHβ subunit knockout mice

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Female mice in which the gene encoding the follicle-stimulating hormone FSH receptor (FSHR) knockout (KO) or its ligand (FSHβKO) have been disrupted were infertile. Ovaries of these mice were significantly smaller than those of heterozygous littermates but significantly larger than those of hypogonadal mice of the same age. Uterine masses in all three mutants were < 6 mg, significantly reduced compared with heterozygous mice. At 1 year of age uterine mass had increased to > 12 mg in 63% of FSHRKO females and 88% of FSHβKO females. Despite the increase in uterine size there was no evidence of contractility: uteri were flaccid and unresponsive to electrical or pharmacological stimulation. In most females in which uterine growth had occurred there was evidence of ovarian growth with hypertrophy of the interstitial tissue, occurrence of ovarian cysts and epithelial and tubular inclusions. There was no evidence of uterine or ovarian hypertrophy in hypogonadal (*hpg*) mice at any age or in 1 year old females in which the FSH mutations were bred onto the *hpg* background. There was an inverse correlation of plasma LH concentrations and uterine mass in 1 year old mutant females with uterine hypertrophy. Ovariectomy of both FSHRKO and FSHβKO females with large uteri resulted in decreased uterine mass and increased plasma concentration of LH. The number of mice with ovarian pathology, reminiscent of the serous ovarian adenocarcinomas found in humans, was significantly greater in the FSHβKO mice, indicating that the presence of an intact FSH receptor on ovarian cells of FSHβKO females may allow constitutive basal stimulation of the ovary, which is absent in mice lacking FSH receptors.

Introduction

Female reproduction is dependent upon an intact hypothalamo–pituitary axis within which cyclic interplay of the gonadotrophic hormones under the control of GnRH stimulates the growth and differentiation of follicles within the ovary eventually resulting in ovulation of selected mature follicles. Follicle-stimulating hormone (FSH), secreted from the pituitary gland, acts on the granulosa cells of the growing follicle via specific receptors (Bousfield *et al.*, 1994) to induce cytochrome P450 aromatase, the enzyme responsible for conversion of androgens from the surrounding thecal cells to oestrogen (Steinkampf *et al.*, 1987; Hickey *et al.*, 1988). Secretion of oestradiol from the ovary is required for growth of the uterus, feedback regulation of gonadotrophin secretion at the pituitary gland and hypothalamus and it may also have a paracrine role within the ovary in the selection of follicles that proceed to ovulation. Female mice that fail to secrete gonadotrophins due to a deletion in the GnRH gene (Cattanach *et al.*, 1977), after gonadotroph ablation, (Kendall *et al.*, 1991), or after disruption of the gene encoding the common alpha subunit (Kendall *et al.*, 1995) have ovaries containing only primordial, primary and pre-antral follicles indicating that gonadotrophins, although not required for follicle recruitment and initial development, are essential for development beyond the pre-antral stage. A similar arrest of folliculogenesis was seen in female mice in which either the gene encoding the FSHβ subunit, responsible for the biological activity of this hormone (Kumar *et al.*, 1997) or the gene encoding the specific receptor for this hormone (Dierich *et al.*, 1998; Abel *et al.*, 2000), had been disrupted, implicating FSH as the specific hormone required at this stage. Similar phenotypes have been reported in women with inactivating mutations in the FSH receptor (FSHR) or FSHβ genes (Themmen and Huhtaniemi, 2000). When the FSHβ knockout (KO) and the FSHRKO mice were produced it was assumed that the resulting phenotypes would be the same and indeed until 8 weeks of age these two mutant strains are phenotypically identical. At 8 weeks of age ovaries

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of both KO females are significantly smaller than those of heterozygous or wild-type littermates and do not contain antral or ovulatory follicles or corpora lutea. Ovarian stromal tissue is hypertrophied and the cells contain numerous small lipid droplets, which is likely to result from exposure to the high circulating concentration of luteinizing hormone (LH) seen in both types of female mutants at this time. The uteri are thin and threadlike in appearance, significantly reduced in mass compared with wild-type mice, reflecting the lack of circulating biologically active oestrogen (Abel *et al.*, 2000). In transgenic mice engineered to produce high concentrations of circulating LH, ovarian pathology is apparent by 4 months of age (Risma *et al.*, 1995), and in women LH hypersecretion has been implicated in infertility, miscarriages and development of granulosa cell tumours (Nilson *et al.*, 2000). FSHβKO and FSHRKO females were maintained for 1 year to investigate any effect upon the ovaries and uteri that might occur with age and to study the underlying endocrine changes related to any changes in pathology. One year old GnRH-deficient hypogonadal (*hpg*) mice in which circulating concentrations of gonadotophins are undetectable have also been examined, together with mice in which the FSHβ and FSHR mutations have been introduced onto the *hpg* genetic background.

Materials and Methods

Mutant mice

FSHR-deficient mice were generated in our laboratory as reported by Abel *et al.* (2000). FSHβ-deficient mice were generated as reported by Kumar *et al.* (1997). We have re-established a breeding colony in our laboratory. *hpg* mice from the original colony developed at the MRC Laboratories, Harwell, Oxford (Cattanach *et al.*, 1977) are bred within our department and the double FSHRKO/*hpg* and FSHβKO/*hpg* mutants were bred by crossing males homozygous for the FSHR and FSHβ deletions with females heterozygous for *hpg*. Male and female double heterozygotes from the F_1 generation were crossed to generate the double mutants in the F_2 generation. Because the FSHRKO and FSHβKO males are fertile, it was possible to introduce FSHKO/heterozygote *hpg* males into the breeding colony as these were identified in the F_2 generation to increase the numbers of double mutants produced. FSHR and FSHβ mutations were identified by Southern blot analysis as described by Abel *et al.* (2000) and Kumar *et al.* (1997) and the *hpg* mutation by PCR analysis as reported by Lang (1991). All animal procedures were carried out under UK Home Office licence.

Histological analysis

Ovaries and uteri were dissected out, weighed and fixed overnight in Bouin's solution, embedded in paraffin wax and sections of 10 μm were stained with haematoxylin and eosin.

Tissue collection

For the analysis of gonadotrophin and steroid hormones blood was collected from the jugular sinus and serum was separated and frozen at −20°C for assay.

Ovariectomy

Under anaesthesia, (Rompun:Ketaset: 0.1 ml kg^{-1} of a 20%:4% (v/v) solution, Veterinary Supplies, University of Oxford), an incision of 0.5 cm was made in the dorsal skin and abdominal wall of the right flank, and the ovary, oviduct and distal region of the uterine horn were exposed. The ovarian artery was located and clamped, and the ovary was dissected free from the surrounding fat and oviduct and removed. Abdominal wall and skin were sutured and the procedure repeated on the left side.

Uterine measurements

One year old FSHβKO and FSHRKO females were subjected to laparotomy; the uterus was visualized and the width measured using an eyepiece graticule. If the size of the uterus was 25 units or greater, bilateral ovariectomy was carried out. Uterine width in 8 weeks old KO females ranged from 2 to 4 units. One week later, mice were anaesthetized, blood was collected and the uterus was removed and weighed.

Epithelial height was measured using an automatic image processing system, Zeiss KS400 (Imaging Associates, Thame). Three measurements were made on three separate fields for each specimen. Mean epithelial height was compared between treatment groups.

Uterine contractility

The uterus was dissected out and placed in ice-cold Ringers solution. Each uterine horn was slit longitudinally; the endometrium was scraped from the underlying myometrium and two strips of myometrium 1 mm wide were isolated and mounted for recording isometric tension in a superfusion organ bath. Tissue was treated with 86 mmol K^+ l^{-1} for 2 min and electrically stimulated at 40 and 60 Hz.

Steroid treatment

Female *hpg* mice, 4–6 months old were implanted subcutaneously in the loose skin at the back of the neck with a 2 cm Silastic implant (Dow Corning Corporation Medical Products, Midland, MI; internal diameter 0.078″) containing either testosterone (Sigma–Aldrich, Poole; T-6147) or oestradiol (Sigma, E-8875). Three weeks later the mice were killed, and their ovaries

and uteri were removed, weighed and fixed in Bouin's solution for histological examination.

Hormone assays

Serum and pituitary concentrations of FSH and LH were measured using in-house immunofluorometric assays (Delfia, Wallac OY, Turku) as described by Haavisto *et al.* (1993) and van Casteren *et al.* (2000). A new pair of antibodies was used in the FSH assay, a monoclonal antibody against recombinant human FSHβ (FSH56A) and a polyclonal antibody against recombinant human FSHα (R93-2705), both donated by Organon (Oss). Sensitivity of the LH assay was 0.75 pg per tube (0.03 $\mu g\,l^{-1}$ in 25 μl); intra-assay coefficient of variation (CV) 19% at 0.04 $\mu g\,l^{-1}$ and < 5% above 1.0 $\mu g\,l^{-1}$; inter-assay CV 12.5% at 0.24 $\mu g\,l^{-1}$ and 7.8% at 0.78 $\mu g\,l^{-1}$. Senstivity of the FSH assay was 0.1 $\mu g\,l^{-1}$, intra-assay CV 4.3% and interassay CV 10.4% at 4.8 $\mu g\,l^{-1}$.

Serum progesterone and testosterone concentrations were measured by radioimmunoassay after diethyl ether extraction as described by Vuorento *et al.* (1989) and Huhtaniemi *et al.* (1985). For serum, sensitivity of the progesterone radioimmunoassay was 0.5 nmol l^{-1}, intra-assay CV 8.0% and interassay CV 9.8% at 1.7 nmol l^{-1} and 2.8 and 4.4%, respectively, at 29 nmol l^{-1}. Sensitivity of the testosterone radioimmunoassay was 1.0 fmol per tube, intra-asssay CV < 6% and interassay CV < 12%.

Statistical analysis

Means were compared by one-way analysis of variance (ANOVA). Where a significant overall difference was detected among groups, differences between individual means were assessed by the Fisher PLSD test. *P* values < 0.05 were considered significant.

Results

*Generation of FSHRKO/*hpg *and FSHβKO/*hpg *double mutants*

The frequency of double mutants identified was 9.4% (16 out of 170) for the FSHRKO/*hpg* mice and 9.7% (11 out of 113) for the FSHβKO/*hpg* mice. The ratio of males to females was 8:8 and 5:6, respectively. With double heterozygote breeders a frequency of 6.3% (1/16) double mutants would be expected in the F_2 generation and with KO/heterozygote males and heterozygote/heterozygote females this would increase to 12.5% (1/8). The frequency of double mutants identified in our colonies reflects the genetic mixture of male breeders in the colony.

Uterine and ovarian masses in 1 year old FSHRKO, FSHβKO, hpg *and double mutant female mice*

The changes in uterine and ovarian masses were evaluated with respect to ageing by analysing the tissues morphologically. Of 79 1 year old FSHRKO females, uterine mass in 63% of animals was >12 mg. Of 49 1 year old FSHβKO females, uterine mass exceeded 12 mg in 88% (Fig. 1). Uterine mass in the remaining females was similar to that seen at 8 weeks of age in both mutants: FSHRKO (5.9 ± 1.9 mg (8)) and FSHβKO (8.6 ± 1.8 mg (8)).

Table 1. Paired ovarian mass in 1 year old FSH receptor (FSHR) and FSHβ heterozygote (H) and knockout (KO) female mice

Mouse	Paired ovarian mass (mg)
FSHRKO (uterine mass < 12 mg)	1.9 ± 0.2 (28) (0.7–4.3)
FSHRKO (uterine mass > 12 mg)	10.4 ± 1.8 (50) (1.7–69.4)
FSHRH	8.2 ± 0.3 (5) (7.6–9.5)
FSHβKO (uterine mass < 12 mg)	4.8 ± 0.6 (6) (2.1–6.2)
FSHβKO (uterine mass > 12 mg)	90.5 ± 39.8 (43) (3.1–1633.9)
FSHβH	11.4 ± 0.9 (9) (6.0–14.6)

Results are shown as the mean ± SEM (*n*) (range).

Growth of the uterus was accompanied by growth of one or occasionally both ovaries (Table 1). There was no increase in either ovarian or uterine mass in *hpg* female mice with age (paired ovarian mass, 0.7 ± 0.1 mg; uterine mass, 5.7 ± 0.5 mg). Similarly in 1 year old double mutant female mice homozygous for the *hpg* and FSHβ or FSH receptor mutations, there was no significant increase in ovarian or uterine mass (paired ovarian mass, 0.8 ± 0.2 mg (6) and 0.8 ± 0.2 mg (6), respectively; uterine mass, 4.1 ± 0.4 mg (6) and 4.3 ± 0.3 mg (6), respectively).

Ovarian and uterine histology

In >90% of ovaries in which there was ovarian pathology there was evidence of structures resembling oviduct epithelium. In some of these mice these inclusions formed large fluid-filled cysts surrounded by a rim of ovarian interstitial and follicular tissue. In a very few ovaries the pathology was different resembling a mass of tubules more reminiscent of kidney within the interstitial follicular tissue (Fig. 2). The height of the uterine epithelium in 8 weeks old female FSHβKO and female FSHRKO and in 1 year old females in which the uterus remained small, was similar to that in *hpg* females. However, the height of the uterine epithelium in both mutants with large uteri was significantly greater than that in *hpg* females ($P < 0.001$). In the *hpg* females treatment with both testosterone and oestrogen increased uterine mass (48.8 ± 1.4 mg (8) (testosterone), 85.1 ± 11.4 mg (6) (oestrogen) compared with 4.6 ± 0.2 mg (18) in the untreated females, and the effect of oestrogen was significantly greater than that of testosterone ($P < 0.001$). Although epithelial height increased with both treatments, the effect of oestrogen was far greater than that of testosterone. Histological analysis of the epithelium in *hpg* females treated with testosterone resembled that seen in the FSHβKO and FSHRKO females

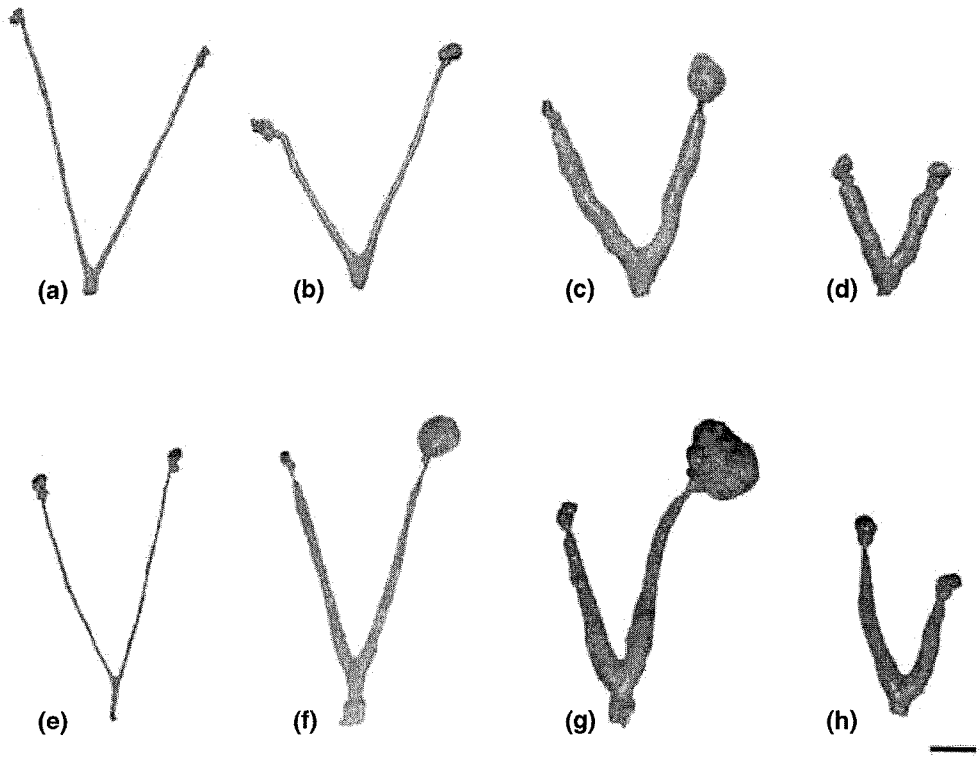

Fig. 1. Gross morphology of the ovaries and uteri of 1 year old FSHR and FSHβ female mice. FSHR knockout (KO): (a) small 37%, (b) medium 31%, (c) large 32% and (d) heterozygote. FSHβKO: (e) small 12%, (f) medium 27%, (g) large 61% and (h) heterozygote. Small, uterus < 12 mg, medium, uterus 12–49 mg, large, uterus > 50 mg. Note the short, thick appearance of the uterus in both heterozgotes due to contraction of the tissue compared with the elongated, flaccid appearance of the uteri in the KO females. Scale bar represents 0.5 cm.

with large uteri, with no significant difference in the height of the uterine epithelium among the mutants and *hpg* females treated with testosterone. However, the height of the uterine epithelium in the mutant females was significantly less than that in the *hpg* females treated with oestrogen ($P < 0.001$).

Serum LH and FSH concentrations in 1 year old FSHR and FSHβ females

There was an inverse relationship between uterine mass and concentrations of LH measured in the serum of 1 year old FSHRKO and FSHβKO females, with the greatest concentrations seen in the mice with small uteri and the lowest in mice showing significant uterine hypertrophy. The difference in serum concentrations of LH between mice with large and small uteri was significant in both FSHRKO and FSHβKO mice ($P < 0.01$ and $P < 0.05$, respectively). Serum FSH showed a similar pattern in the FSHRKO females. Serum concentrations of LH and FSH in the FSHRKO females and LH in the FSHβKO females were higher than concentrations measured in randomly cyclic heterozygote females of the same age (Fig. 3).

Effect of ovariectomy on uterine mass and serum gonadotrophin concentrations

Uterine mass. The mean uterine mass measured after ovariectomy in FSHRKO females was 11.5 ± 2.6 mg ($n = 5$) and in FSHβKO females was 23.7 ± 1.2 mg ($n = 10$), which is significantly lower than the uterine mass in KO females in the combined medium-to-large uterine categories (FSHRKO, 64.1 ± 15.1 mg (9); and FSHβKO, 106.4 ± 24.5 mg (10); $P < 0.05$ and $P < 0.01$, respectively).

Serum gonadotrophin concentrations. Serum LH concentrations rose in both the ovariectomized FSHRKO females (3.2 ± 0.2 ng ml^{-1} (5)) and FSHβKO females (3.0 ± 0.2 ng ml^{-1} (9)) to reach concentrations not significantly different from those seen in the mice showing no uterine hypertrophy (4.0 ± 1.1 (5) ng ml^{-1}, FSHRKO and 3.2 ± 1.7 (4) ng ml^{-1}, FSHβKO).

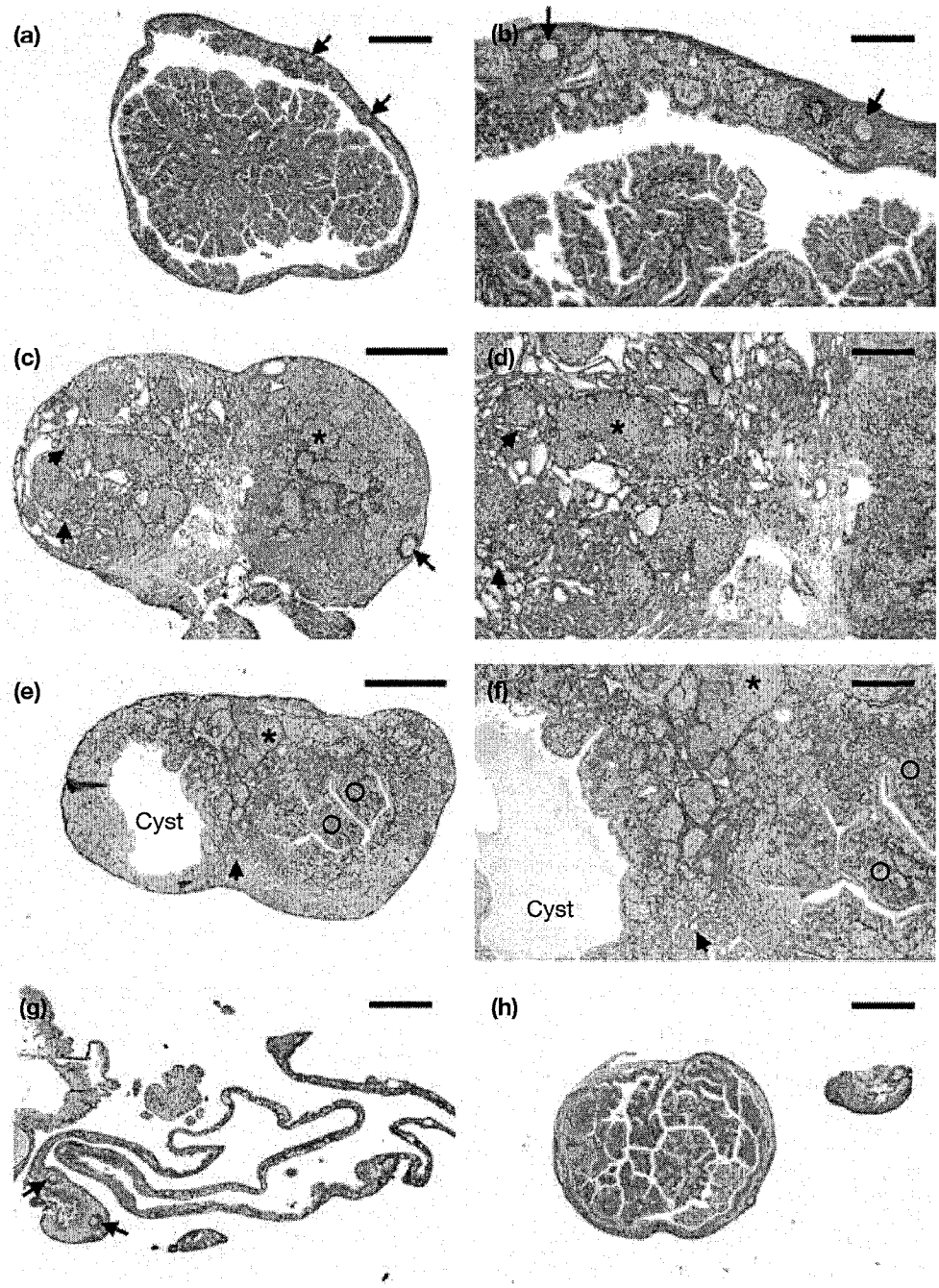

Fig. 2. Sections through the ovaries of 1 year old FSHR knockout (KO) and FSHβKO female mice with large uteri. (a,b) FSHRKO: cyst-like structure with remnants of ovarian tissue on the inner surface of the ovarian membrane (arrows indicate ovarian follicles) and epithelial lined oviduct-like structures in the central region, reminiscent of the human serous ovarian adenocarcinomas described by Auersperg *et al.* (2001). (c,d) FSHRKO: enlarged ovary showing ovarian follicle (long arrow) with hypertrophied interstitial tissue on right (asterisk) and tubular structures interspersed between foci of interstitial tissue on the left (short arrow). (e,f) FSHRKO: enlarged ovary with cyst forming on left surrounded by hypertrophied interstitial–stromal tissue (asterisk), oviduct-like structures (O) and tubular structures (short arrow). (g) FSHβKO: a collapsed cyst with remnants of ovarian tissue attached (arrows indicate ovarian follicles). (h) Comparison of ovaries from the same FSHRKO mouse. Left: enlarged and showing oviduct-like structures; right: small ovary, no different in appearance from ovaries of 1 year old mutant females showing no uterine hypertrophy. Scale bars represent 200 μm (b,d,f), 400 μm (c,e) and 500 μm (a,g,h).

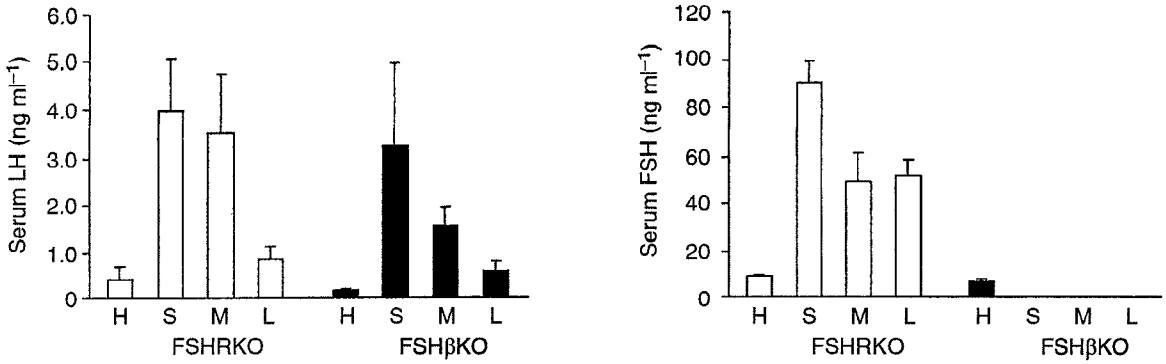

Fig. 3. Serum LH and FSH concentrations in 1 year old FSH receptor (FSHR) (□) and FSHβ (■) heterozygote (H) and knockout (KO) female mice. The KO females are subdivided according to uterine mass into small (S), uterus < 12 mg; medium (M), uterus 12–49 mg; and large (L), uterus > 50 mg. Results are expressed as the mean ± SEM. There is a mimimum of four mice in each group.

Table 2. Serum progesterone and testosterone concentrations in 1 year old FSH receptor (FSHR) and FSHβ heterozygote (H) and knockout (KO) female mice

Mouse	Progesterone (nmol l^{-1})	Testosterone (nmol l^{-1})
FSHRH	17.6 ± 6.2 (5)	0.3 ± 0.1 (2)
FSHRKO-S	8.9 ± 5.7 (5)	1.2 ± 0.9 (3)
FSHRKO-M	10.7 ± 3.8 (5)	1.8 ± 0.9 (4)
FSHRKO-L	4.4 ± 3.0 (5)	1.4 ± 0.5 (3)
FSHβH	12.8 ± 4.1 (9)	1.0 ± 0.5 (5)
FSHβKO-S	2.5 ± 0.5 (4)	0.6 ± 0.2 (4)
FSHβKO-M	5.3 ± 2.3 (5)	1.6 ± 0.4 (5)
FSHβKO-L	3.7 ± 0.9 (5)	0.4 ± 0.1 (4)

KO mice are subdivided by uterine mass into small (S, < 12 mg), medium (M, 12–49 mg) and large (L, > 50 mg). Results are shown as the mean ± SEM (*n*).

Serum steroid concentrations in 1 year old FSHR and FSHβ female mice

Serum progesterone concentrations in all FSHRKO and FSHβKO 1 year old females were lower but not significantly so compared with concentrations measured in heterozygote females. In contrast, serum testosterone concentrations were higher than in heterozygote females in all FSHRKO females and in FSHβKO females with moderate uterine hypertrophy. Again differences were not significant in part due to the low sample number, as serum samples had to be pooled in order to carry out testosterone measurements (Table 2).

Uterine contractility

Uterine strips from four heterozygote females exhibited spontaneous uterine contractions *in vitro*. Hyperpolarization was induced by potassium followed by a period of depolarization. Electrical stimulation induced an immediate contraction comparable in amplitude to the spontaneous contractions. In contrast, uterine tissue from FSHRKO and FSHβKO females showed no spontaneous contractile activity *in vitro* and no response to either potassium or electrical stimulation (data not shown).

Discussion

The exact causes of the ovarian hypertrophy and eventual tumour formation that occur in most FSH receptor- and FSHβ-deficient mice at 1 year of age remains unknown. The fact that there was no pathology in the 1 year old *hpg* females in which serum concentrations of LH and FSH are extremely low, nor in females in which either mutation was introduced onto the *hpg* background, indicates that the major driving force is gonadotrophic hormone stimulation of the ovary. Since FSH is absent in the FSHβKO females and the FSH receptor is not present in the FSHRKO females, chronic exposure to high concentrations of LH would appear to be the primary cause of the observed pathology. In transgenic mice expressing LHβ (Risma *et al.*, 1995, Nilson *et al.*, 2000) or hCGβ (Rulli *et al.*, 2002) transgene, the increased plasma LH concentrations have been implicated in the initiation of the subsequent ovarian pathology. Conversely, preliminary observations in five 1 year old female mice in which the LH receptor has been disrupted (Zhang *et al.*, 2001) show no such ovarian pathology (data not shown).

Ovarian tumours in species other than humans arise in follicular, stromal or germ cells rather than in the ovarian surface epithelium, and the biololgy of these tumours is fundamentally different from that of epithelial ovarian cancer. However the histological appearance of the enlarged ovaries reported in this study resembles that of the human epithelial ovarian carcinomas described by Auersperg *et al.* (2001), in particular those with an oviduct-like epithelial appearance, classified as serous ovarian adenocarcinoma. The appearance contrasts with the mixed cell type tumours reported by Matzuk *et al.* (1992) in their inhibin-deficient female mice.

Although the ovaries of both FSHβKO and FSHRKO female mice are significantly larger than ovaries of *hpg* females at 8 weeks of age, they are much smaller than ovaries of heterozygote or normal females. Growth of follicles to full preovulatory stages does not occur. However, Burns *et al.* (2001) described a well formed thecal layer in their FSHβKO females with evidence of 17α hydroxylase activity and LH receptor mRNA from 6–16 weeks of age, indicating that thecal–interstitial tissue formation and function is independent of FSH. Semi-thin sections through the ovary of FSHRKO females at 8 weeks of age show stromal cells containing large numbers of small lipid droplets, indicating that this tissue is steroidogenically active (Abel *et al.*, 2000). It is likely that this is a response to the high circulating concentrations of LH measured in these mice at this age.

The presence of a defined thecal layer indicates that androgen production by these cells is likely to be occurring in response to endogenous LH production. In the absence of FSH stimulation it is unlikely that there would be significant aromatase activity within these ovaries to generate oestrogen despite the availability of substrate. In support of this contention, Burns *et al.* (2001) reported a decrease in P450 aromatase mRNA in FSHβKO females up to 4 months of age. The histology of the uterine epithelium in the mutants with hypertrophy mirrors that of the *hpg* females treated with testosterone, and serum testosterone concentrations measured in the mutants indicate that ovarian secretion of this steroid could be the cause of uterine growth. Although the uterus increases in mass with age, the tissue remains flaccid and non-contractile supporting the idea that oestrogen is not produced in any significant amount in the ovaries of these mice since development of the contractile proteins within the smooth muscle cells of the uterus is dependent upon oestrogen stimulation (Csapo and Corner, 1952; Michael and Scholfield, 1967). In the oestrogen receptor α KO female there is evidence that ovary-derived androgens provide some maintenance of uterine mass that is lost on ovariectomy (Lindzey and Korach, 1997). In transgenic mice expressing a chimaeric LHβ subunit, resulting in a five- to tenfold increase in plasma LH concentrations, superimposed on a normal FSH background, there is an increase in both testosterone and oestrogen with an increase in the testosterone : oestrogen ratio (Risma *et al.*, 1995). Surprisingly, Danilovich *et al.* (2000) reported a tenfold increase in serum testosterone concentrations in their FSH receptor knockout (FORKO) mice at 3–4 months of age but despite this increase in circulating testosterone no increase in uterine growth was found. Despite the absence of biologically active oestrogen in our mutant females we have not found any overt skeletal abnormalities in 1 year old animals, which contrasts with the marked kyphosis reported by Danilovich *et al.* (2000) in their FORKO females.

Although overstimulation of the thecal–interstitial tissue to produce steroids can explain the observed uterine growth, what causes the altered differentiation of the ovarian tissue to form tumours remains to be determined. It is possible that local high concentrations of steroids within the ovary could be causative. It is also possible that constant high amounts of LH could directly activate local growth factors, and that androgen production is merely a by-product of thecal stimulation. The resemblance of most of the tumours seen in this study to the epithelial ovarian carcinomas found in humans (Auersperg *et al.*, 2001) raises the possibility that the surface epithelium of the ovaries in the mutant females may undergo stimulation in response to the high serum concentrations of LH. Proliferation of this tissue in response to hCG has been shown in mice *in vitro*, indicating that receptors for LH/hCG are present on the surface epithelium (Davies *et al.*, 1999) and recent research in sheep has shown an active involvement of this tissue in the mechanism of ovulation after the LH surge (Murdoch and McDonnel, 2002). Activin has been shown to stimulate proliferation of ovarian thecal–interstitial tissue in rats (Duleba *et al.*, 2001) but low amounts of activin subunit mRNAs were found in granulosa cells in 8 weeks old FSHβ-deficient mice (Burns *et al.*, 2001), indicating that activin is not a major factor in the ovarian hypertrophy that develops in these mutant females. However, inhibin has been proposed to be a gonadal tumour suppressor (Matzuk *et al.*, 1992) and low amounts of this hormone in the absence of FSH stimulation (Turner *et al.*, 1989) could be permissive for tumour development within the ovary. A comparison of ovarian gene expression among ovaries removed from female mutants at the onset of pathological change, as indicated by an early, significant increase in uterine growth, might provide a means for dissecting out differences correlated with tumour differentiation.

Serum LH concentration remained high in female mice that showed no growth of the uterus at 1 year of age; concentrations were similar to those seen in 8 weeks old mutant females (Kumar *et al.*, 1997, Abel *et al.*, 2000). In contrast, where uterine hypertrophy occurred, serum LH concentrations showed an inverse correlation with uterine mass. Ovariectomy of 1 year old mice with enlarged uteri resulted in a significant decrease in uterine mass and increase in serum LH concentrations within 1 week, supporting the theory that substances produced by the ovary were responsible for the observed uterine hypertrophy.

The reason for the greater number of FSHβKO mice developing uterine and ovarian hypertrophy is unclear. It might be expected that disruption of the gene encoding the FSHβ ligand would produce the same phenotype as disruption of the gene encoding the receptor for this ligand, since this hormone acts through a single receptor type. Indeed the phenotypes of the FSHβKO and FSHRKO female mice at 8 weeks of age are identical

but there is now evidence that by 10 weeks a number of FSHβKO females begin to show an increase in both ovarian and uterine mass. This increase is not seen in FSHRKO females until approximately 4–6 months of age and the difference between FSHβKO and FSHRKO females becomes more marked with age. The major difference between these two KO mice at the ovary is the presence of a functional FSH receptor in the FSHβKO females. Activating mutations of both the FSH and LH receptors have been reported (Themmen and Huhtaniemi, 2000) and there is evidence that G-protein-coupled receptors exhibit various degrees of constitutive activity in the absence of ligand (de Ligt *et al.*, 2000). Transfection of the wild-type FSH receptor into MSC-1 cells *in vitro* induced a significant increase in cAMP concentration compared with cells transfected with the vector alone (Baker *et al.*, 2003) supporting the idea that the FSH receptor possesses activity in the absence of binding of the FSH ligand *in vivo*.

In older females, fewer follicles and granulosa cells are present in the ovary compared with young mice. Therefore, any constitutive activity of the FSH receptor in the FSHβKO females is likely to be occurring in younger females in which follicles up to the pre-antral stage are present. This activity may enhance the action of LH within the ovary and accelerate the production of factors responsible for the observed ovarian and uterine hypertrophy in the FSHβKO females. Ligand-induced reduction in cellular concentrations of receptor mRNA has been described for the LH receptor (Segaloff *et al.*, 1990; Hoffman *et al.*, 1991) and for the FSH receptor in cultured rat granulosa cells (Minegishi *et al.*, 1995). In addition, desensitization of the FSH receptor occurs after prolonged exposure to ligand (for review, see Simoni *et al.*, 1997). In the absence of hormone it might be expected that receptor activity was higher than normal and interestingly in FSHβKO mice up to 4 months of age, Burns *et al.* (2001) found an increased amount of FSHR mRNA relative to normal mice of the same age. Thus, any constitutive activity of the FSH receptor in the absence of ligand is likely to be enhanced in the younger FSHβKO females and be a further factor enhancing pathological changes in the ovaries of these mice compared with the FSHRKO females. An additional explanation would be that a factor or factors produced in response to LH can activate the FSH receptor and turn on downstream pathways. What is clear from this study is that prolonged exposure to high concentrations of LH in the absence of FSH results in pathological changes within the ovary leading to subsequent tumour formation and uterine hypertrophy.

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