REPRODUCTION
REVIEW

Focus on Gonadotrophin Signalling

What have gonadotrophin overexpressing transgenic mice taught us about gonadal function?

Susana B Rulli[1] and Ilpo Huhtaniemi[2]

[1]*Institute of Biology and Experimental Medicine-CONICET, Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina and* [2]*Institute of Reproductive and Developmental Biology, Imperial College Faculty of Medicine, Hammersmith Campus, Du Cane Road, London W12 0NN, UK*

Correspondence should be addressed to I Huhtaniemi; Email: ilpo.huhtaniemi@imperial.ac.uk

Abstract

The two gonadotrophins, follicle-stimulating hormone and luteinising hormone, are pivotal regulators of the development and maintenance of normal fertility by maintaining testicular and ovarian endocrine function and gametogenesis. Too low gonadotrophin secretion, i.e. hypogonadotrophic hypogonadism, is a common cause of infertility. But there are also physiological and pathophysiological conditions where gonadotrophin secretion and/or action are either transiently or chronically elevated, such as pregnancy, pituitary tumours, polycystic ovarian syndrome, activating gonadotrophin receptor mutations, perimenopause and menopause. These situations can be either the primary or secondary cause of infertility and gonadal pathologies in both sexes. Also the role of gonadotrophins as tumour promoters is possible. Recently, the possibility to combine information from genetically modified mice and human phenotypes in connection with mutations of gonadotrophin or gonadotrophin receptor genes has elucidated many less well known mechanisms involved in dysregulation of gonadotrophin function. Among the genetically modified mouse models, transgenic mice with gonadotrophin hypersecretion have been developed during the last few years. In this review, we describe the key findings on transgenic mouse models overexpressing gonadotrophins and present their possible implications in related human pathologies. In addition, we provide examples of genetic mouse models with secondary effects on gonadotrophin production and, consequently, on gonadal function.
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Physiological actions of gonadotrophins

Gonadal function is regulated by complex physiological and molecular processes that are to a great extent under the control of the hypothalamic–pituitary–gonadal axis. The integrity of this regulatory cascade is crucial, since disturbances at any of its levels can produce reproductive defects. The pituitary gonadotrophins follicle-stimulating hormone (FSH) and luteinising hormone (LH) and their receptors (R) are the main regulators of gonadal function, and have been extensively studied (Simoni *et al.* 1997, Ascoli *et al.* 2002, Burger *et al.* 2004). LH and FSH, together with thyroid-stimulating hormone, are members of the pituitary glycoprotein hormone family. They are heterodimers composed of a common α-subunit and the hormone-specific β-subunit. LH and FSH exert their effects through binding to their cognate G-protein-coupled transmembrane receptors (GPCR; Simoni *et al.* 1997, Ascoli *et al.* 2002).

The decapeptide gonadotrophin-releasing hormone (GnRH) is released in a pulsatile fashion from specific hypothalamic nuclei into the hypothalamo–hypophyseal portal circulation, and it stimulates the synthesis and secretion of LH and FSH. Gonadal steroid and peptide (mainly inhibin) hormones exert negative and positive feedback effects on gonadotrophin synthesis and secretion, either directly at the pituitary level or indirectly via the hypothalamus, mainly by modulating GnRH secretion (Burger *et al.* 2004). The physiological actions of LH and FSH are well characterised and they are essential for folliculogenesis, ovulation and steroidogenesis in females, and for testicular growth, spermatogenesis and steroidogenesis in males. The placental analogue of LH, human chorionic gonadotrophin (hCG), is a fourth member of the glycoprotein hormone family. However, it interacts with the same LH/hCG receptor as LH, and functions as an LH agonist with a longer half-life and higher biopotency than its pituitary counterpart (Jameson & Hollenberg 1993).

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The first experimental evidence suggesting that the gonadal function is regulated by pituitary-derived compound(s) was based on implantation of the anterior pituitary gland into immature male and female rodents, which induced precocious puberty, enlargement of the ovaries and superovulation (Smith & Engle 1927). A few years later, the gonadotrophic principle was proposed, due to the extraction from the pituitary gland of two different hormones that both stimulated the gonads, displaying biological properties of the follicle-stimulating and luteinising hormones respectively as we know them today (Fevold *et al.* 1931). With regard to hCG, it was demonstrated that the blood and urine of pregnant women presented with gonad-stimulating properties, originating from the placenta.

Subsequently, the isolation of increasingly purer gonadotrophin preparations from pituitary glands for experimental studies and from urinary extracts for clinical use has advanced our knowledge of gonadotrophin physiology, and improved the management of infertility. However, it is difficult to develop protocols for chronic administration of exogenous gonadotrophins that would mimic physiological effects of gonadotrophin secretion in the long term. Since these methods rely on pharmacological strategies to elevate gonadotrophin levels, findings on short- and long-term gonadotrophin treatments in experimental animals seem to be strongly age, dose, and time dependent (Risbridger *et al.* 1982, Scott *et al.* 1990). Moreover, formation of antibodies against heterologous proteins poses a confounding factor upon long-term experiments. In most experimental conditions, treatment of animals chronically with LH, hCG, or FSH results in decreased responses of their testicular and ovarian target cells through receptor down-regulation and desensitisation of signalling (Conti *et al.* 1976, Dufau *et al.* 1979).

The advent of recombinant DNA technology and transgenic animals made it possible to obtain new insight into the effects of chronically elevated gonadotrophin levels and the physiopathology of function of the hypothalamic–pituitary–gonadal axis. Consequently, mouse models exist now for many human reproductive abnormalities due to genetic alterations in gonadotrophin secretion or action (Themmen & Huhtaniemi 2000, Huhtaniemi & Themmen 2005, Themmen *et al.* 2005). Despite the major advancement in this field, many reproductive disturbances still remain idiopathic.

Elevated gonadotrophin levels and human diseases

There are different physiological and pathophysiological conditions where gonadotrophins can be either transiently or chronically elevated during the lifetime of humans. One such situation is pregnancy when hCG is produced in very high amounts during the first trimester to maintain the progesterone production of the corpus luteum of pregnancy, which prepares the uterus for implantation, and for embryonic and placental development (Jameson & Hollenberg 1993). hCG also stimulates foetal testicular testosterone production which is needed for masculinisation of the male foetus.

Another physiological condition with highly elevated gonadotrophin levels occurs in women during ageing. Gonadotrophin secretion starts slowly increasing during the years preceding the menopause (premenopause), and increases 10- to 20-fold after the last menstrual bleeding, menopause. Thereafter, the female body is chronically exposed to high levels of gonadotrophins for decades. The postmenopausal increase in gonadotrophin levels is basically due to exhaustion of the ovarian follicle pool and consequent cessation of the negative feedback of ovarian hormones (oestrogen, progesterone, inhibin) at the hypothalamic–pituitary level. Despite the cessation of ovarian function, this prolonged exposure to gonadotrophins may have effects on postmenopausal women, especially in view of the ubiquitous extragonadal expression of LHR/hCGR (Filicori *et al.* 2005).

In terms of pathological conditions, surgical or chemical gonadectomy (Huirne & Lambalk 2001), primary gonadal failure with missing feedback regulation (Salbenblatt *et al.* 1985, Quigley 2002), hypothalamic dysfunction (Marshall *et al.* 2001), or gonadotroph adenomas (Roberts *et al.* 2005) are other known conditions that induce high gonadotrophin levels. On the other hand, expression of hCG occurs in trophoblastic diseases and testicular germ cell tumours (Cole & Butler 2002, Stenman *et al.* 2004). Finally, polycystic ovarian syndrome (PCOS) is a condition where chronic elevation of LH levels is a diagnostic hallmark (Franks & McCarthy 2004).

Many clinical and epidemiological studies have suggested that inappropriately elevated gonadotrophin action leads to infertility and gonadal pathologies in both sexes. For instance, elevated gonadotrophin levels are associated with ovarian hyperstimulation syndrome (Delvigne & Rozenberg 2002) and PCOS (Franks & McCarthy 2004). Exposure to elevated gonadotrophins after the menopause or infertility treatments is proposed to be a risk factor for developing ovarian tumours (Risch 1998, Konishi *et al.* 1999, Riman *et al.* 2004). In contrast, reduced risk for ovarian cancer is associated with multiple pregnancies, breast-feeding, oral contraceptive use, and oestrogen replacement therapy, all of which lead to lower levels and reduced exposure to gonadotrophins (Gnagy *et al.* 2000, La Vecchia 2001). Much less is known about possible pathological effects of gonadotrophin dysregulation in men, and on the basis of available evidence it seems that the female reproductive system is more vulnerable to gonadotrophin dysregulation than the male.

In men, the best examples of reproductive disturbances due to defective gonadotrophin action are the naturally occurring mutations of LHR and FSHR (Themmen & Huhtaniemi 2000, Huhtaniemi & Themmen 2005). The activating mutations permanently stimulate, in the absence of their cognate ligand, the receptor signalling pathways, evoking a condition of precocious, chronically elevated gonadotrophin action. In the case of LHR, a number of

activating mutations causing male-limited gonadotrophin-independent hypersecretion of testosterone and precocious puberty have been described (Themmen & Huhtaniemi 2000). Interestingly, one specific mutation ($Asp^{578}His$) has been reported in association with Leydig cell adenomas (Liu *et al.* 1999). This observation supports the hypothesis that gonadotrophin action is involved in gonadal tumorigenesis. On the other hand, only one activating mutation of the FSHR gene has so far been identified in humans (Gromoll *et al.* 1996). This patient, a male who was hypophysectomised and treated with testosterone, displayed normal spermatogenesis and was, unexpectedly, fertile in spite of undetectable gonadotrophins.

Interestingly, FSHR mutations making the receptor responsive to hCG stimulation were recently identified by two different groups in association with familial gestational spontaneous ovarian hyperstimulation syndrome (Smits *et al.* 2003, Vasseur *et al.* 2003). These mutations display abnormal hypersensitivity to hCG activation through the structurally altered FSHR, which presented during pregnancy when the circulating levels of endogenous hCG are very high. This finding supports the concept that the FSH pathway has a pivotal role in the pathophysiology of ovarian hyperstimulation syndrome.

Although gonadotrophin actions are mainly directed to the regulation of gonadal function, the recently discovered extragonadal sites of gonadotrophin subunit and receptor expression suggest that extragonadal gonadotrophin effects may also exist (Filicori *et al.* 2005). This possibility extends the spectrum of gonadotrophin actions, especially in women, beyond reproductive age and suggests the possibility that the high postmenopausal gonadotrophin levels could have a physiological role. However, the topic of extragonadal gonadotrophin action remains controversial, not the least in light of the very recent findings on LHR knockout mice (Pakarainen *et al.* 2005).

Transgenic mouse models as a tool to study gonadal function

The generation of genetically modified animal models provides powerful means to understand the physiological role of gonadotrophins in reproductive function as well as the pathologies arising from their dysregulation. These techniques provide *in vivo* models to study the role of a particular hormone throughout the life of the animal, including the very early developmental stages. Increasing interest has emerged among researchers to generate animal models that mimic human pathologies (Burns & Matzuk 2002, Huhtaniemi *et al.* 2002, 2005). Gain-of-function models consisting of mice overexpressing a certain gene of interest may mimic the effects of hypersecretion syndromes and activating mutations in humans. Another alternative is the generation of mice bearing targeted point mutations that may be even closer phenocopies of human activating mutations. On the other hand, loss-of-function mutations in knockout mice are able to recreate hormone deficiency and resistance syndromes in humans (Kumar *et al.* 2005). We will present below key examples of transgenic mice overexpressing gonadotrophins and their possible implications in relation to human pathologies (Table 1).

Transgenic mice overexpressing FSH

In order to elucidate the biological role of FSH in gonadal growth, function and tumour development, Kumar *et al.* (1999) developed gain-of-function mutant bi-transgenic mice overexpressing the human glycoprotein hormone-α and human (h) FSHβ-subunits under the ectopically expressing mouse metallothionein-1 promoter. With this approach, one line of mice with a low copy number of hFSHβ directed the expression of circulating hFSH at levels comparable to those in postmenopausal women. In this case, both males and females were fertile and did not present any abnormalities in other tissues. These studies showed that prolonged exposure to elevated FSH levels for more than one year did not directly cause ovarian tumorigenesis or other functional abnormalities.

A second line of these transgenic mice had a high copy number of the FSHβ transgene, expressing hFSH levels that by far exceeded those found in postmenopausal women (Kumar *et al.* 1999). Female transgenic mice were infertile and developed haemorrhagic and cystic ovaries. They had elevated testosterone, oestradiol and progesterone levels in serum, and developed enlarged and cystic kidneys. These mice died before 13 weeks of age due to urinary tract obstruction, but had no signs of ovarian tumours. This concept was further confirmed with the generation of mouse models with different genetic approaches that supported the influence of FSH as an important trophic modifier for gonadal tumorigenesis. Inhibin-deficient mice that have increased FSH and activin levels developed multiple sex-cord stromal tumours of the granulosa and Sertoli cell lineage (Matzuk *et al.* 1992). It was demonstrated that gonadotrophins are required for tumour development in these mice, since double-mutant mice homozygous for the gonadotrophin-deficient hypogonadal (*hpg*) mutation in the inhibin null background did not develop tumours (Kumar *et al.* 1996). Moreover, inhibin and FSH null mice showed a significant delay in tumour development and a less aggressive phenotype compared with mice deficient only in inhibin (Kumar *et al.* 1999). A similar role for gonadotrophins as tumour promoters was demonstrated in transgenic mice expressing the SV40 T-antigen under the inhibin α-subunit promoter, since development of gonadal somatic cell tumours in these mice was dependent on gonadotrophin secretion (Kananen *et al.* 1997, Mikola *et al.* 2003). However, in these mice it was found that LH was the main tumour-promoting gonadotrophin.

Male hFSH transgenic mice were infertile, produced elevated levels of serum testosterone and presented with enlarged seminal vesicles. Testicular growth and spermatogenesis, however, appeared morphologically normal.

Table 1 Transgenic mouse models with gonadotrophin overexpression.

Construct	Phenotype	References
MT-1 promoter/human FSHαβ	Males infertile, normal spermatogenesis, reproductive tract obstruction; females infertile, haemorrhagic and cystic ovaries	Kumar *et al.* (1999)
Rat insulin II promoter/FSHαβ × *hpg*	Selective FSH action independently of LH	Allan *et al.* (2004)
Rat androgen-binding protein promoter/ mutant FSH receptor × *hpg*	Constitutive signalling and cell-surface expression of FSH receptor; male specific; FSH-like Sertoli cell response	Allan *et al.* (2004)
Inhibin α knockout	High FSH and activin; sex-cord stromal tumours	Matzuk *et al.* (1992)
Inhibin α knockout × *hpg*	Sex-cord stromal tumours abolished	Kumar *et al.* (1996)
Inhibin α knockout × FSH knockout	Sex-cord stromal tumours delayed	Kumar *et al.* (1999)
hCGβ promoter/hCGβ cluster	No phenotype	Strauss *et al.* (1994)
bLHα promoter/bLHβ-CTP	Males fertile; females polycystic ovaries, granulosa/theca cell tumours, pituitary adenomas, mammary tumours	Risma *et al.* (1995), Mann *et al.* (2003)
Ubiquitin C promoter/hCGβ	Males fertile; females infertile, ovaries luteinised, prolactinomas, metastatic mammary adenocarcinomas	Rulli *et al.* (2002)
Ubiquitin C promoter/hCGαβ	Males infertile, foetal Leydig cell adenomas, urethral and kidney defects; females infertile, ovarian teratomas	Rulli *et al.* (2003), Huhtaniemi *et al.* (2005), Ahtiainen *et al.* (2005)
MT-1 promoter/hCGβ	Males and females infertile; cystic and haemorrhagic ovaries	Matzuk *et al.* (2003)
MT-1 promoter/hCGαβ	Males infertile, Leydig cell hyperplasia; females infertile, cystic and haemorrhagic ovaries, cystic kidneys	Matzuk *et al.* (2003)
hCG-LH receptor complex	Male and female gonadal defects; altered hormone levels	Meehan *et al.* (2005)
Estrogen α receptor knockout	Males infertile, seminiferous tubules disrupted; females infertile, high LH; cystic and haemorrhagic ovaries	Schomberg *et al.* (1999)
Estrogen αβ receptor knockout	High LH; males infertile; females infertile, follicle transdiferentiation to seminiferous tubules	Couse *et al.* (1999*b*)
Aromatase knockout	High testosterone, LH and FSH; males progressively infertile; females infertile, disrupted folliculogenesis	Britt *et al.* (2001)

MT-1, metallothionein 1.

The infertility of these mice was probably due to an obstruction in the ejaculatory tract that prevented the access of epididymal sperm to semen (Kumar *et al.* 1999).

The results obtained from the FSH overexpressing female mice resemble known human reproductive pathologies, in which ovarian cyst formation and haemorrhage are often associated with ovarian cancer in postmenopausal women or in patients with ovarian hyperstimulation syndrome. In contrast to females, there is no direct evidence that inadvertently elevated FSH levels can affect male fertility in humans.

In this respect, in an attempt to study the specific role of FSH in gonadal function independently of LH activity, a series of genetic mouse models was created by combining the gonadotrophin-deficient background of *hpg* mice with transgenic mice expressing the heterodimeric FSH, or a mutant human FSH receptor containing a single amino acid substitution ($Asp^{567}Gly$) equivalent to activating mutations in related glycoprotein hormone receptors (Allan *et al.* 2004). These findings revealed that full Sertoli cell proliferation could be accomplished by FSH activity without LH. There were no obvious gonadal phenotypes on the normal mouse background, suggesting that, in these models, the transgene expression did not exceed the physiological FSH response.

Transgenic mice overexpressing LH

To address the impact of chronically elevated LH action on the reproductive system, different animal models with chronic hypersecretion of LH or hCG have been developed. Transgenic LHβCTP mice first reported by Risma *et al.* (1995) expressed a fusion gene of the coding sequence of bovine (b) LHβ subunit fused in-frame with the C-terminal peptide sequence of the human chorionic gonadotrophin-β subunit (CTP), driven by the pituitary-specific bovine glycoprotein-α subunit promoter. This transgene was targeted to the pituitary gonadotroph cells, and it achieved physiological levels of LH in the circulation, ranging from a 5- to 10-fold increase in females, but no apparent LH elevation occurred in transgenic males. Female LHβCTP mice presented with precocious puberty (Risma *et al.* 1997), and suffered from accelerated depletion of primordial follicles in the ovary (Flaws *et al.* 1997). These mice are infertile primarily due to chronic anovulation (Risma *et al.* 1995), which can be reversed by administration of an LH surge (Mann *et al.* 1999). In addition to this, the hormone imbalance of the LHβCTP females produces defects in uterine receptivity and induces mid-gestation pregnancy failure (Mann *et al.* 1999). Since these mice develop enlarged ovaries containing multiple cysts producing increased levels of testosterone and oestradiol, chronic hypersecretion of LH in the LHβCTP mice was proposed as a useful model to recapitulate PCOS in humans (Risma *et al.* 1995, Mann *et al.* 2003).

The majority of human ovarian tumours are epithelial in origin, to a lesser extent they are of sex-cord stromal or germ cell origin, and granulosa cell tumours are very rare (Amsterdam & Selvaraj 1997). Gonadotrophin hypersecretion has long been implicated in ovarian tumour development, especially based on epidemiological studies (Risch

1998), and recently, these data have been supported by studies in experimental animal models (Risma *et al.* 1995, Kananen *et al.* 1997, Kumar *et al.* 1999). In this respect, the LHβCTP female mice occasionally develop granulosa cell tumours in old age (Risma *et al.* 1995). However, it was later demonstrated that the nature of ovarian tumour formation was directly dependent on the genetic background of the mouse strain used for breeding, and this feature, i.e. the need for a specific genetic constellation, may also contribute to the rarity of these tumours in women. For instance, in the CF-1 outbred strain, elevated LH causes granulosa cell tumours by 5 months of age. It was suggested that the susceptibility to granulosa cell tumours is an oligogenic trait controlled by three unlinked genes (Keri *et al.* 2000). Hybrid mice, instead, displayed cystic ovaries with a highly luteinised phenotype reminiscent of luteomas of pregnancy (Keri *et al.* 2000). Interestingly, the same ovarian phenotype was developed when LHβCTP (CF-1) mice were treated with repetitive hCG surges, indicating that the ovulatory LH/hCG surges are able to prevent granulosa cell tumours, independently of the mouse strain used (Owens *et al.* 2002).

Kero *et al.* (2000) demonstrated that chronically elevated LH in female LHβCTP mice causes adrenal hyperplasia, and induces LHR expression in the adrenal gland, together with stimulation of corticosterone production. This effect appears to be dependent on ovarian hyperfunction, probably due to its polycystic condition, since gonadectomy abolishes the adrenal disturbances. These mice have a phenotype reminiscent of Cushing's syndrome, and they may provide a useful model to study human adrenal hyperfunction associated with chronically elevated gonadotrophin secretion, such as occurs in postmenopausal women or in those with PCOS.

Transgenic mice overexpressing hCG

Many endocrine disorders are attributed to excessive secretion of the hormone beyond physiological levels. Consequently, we have recently developed a transgenic mouse model that secretes pharmacological levels of hCG, although compared with humans not exceeding those occurring in pregnancy (Rulli *et al.* 2002, 2003). By intentionally exaggerating hCG production, we were able to recognise novel phenotypes both in males and females that could not be revealed by models displaying more moderate elevation of hormone secretion.

We first generated a transgenic mouse model bearing the hCGβ subunit under the human ubiquitin promoter (hCGβ +), in which hCG is moderately overproduced in a large number of tissues (Rulli *et al.* 2002, 2003). By association with the endogenously expressed common α-subunit in the pituitary gland, bioactive hCG α/β dimers were produced and secreted into the circulation, achieving around a 40-fold increase in LH/hCG bioactivity in female animals (Rulli *et al.* 2002), but only a 3- to 4-fold increase in males (Rulli *et al.* 2003). In this model, the availability of the endogenous α-subunit becomes rate limiting in the dimerisation process of hCG, since the hCGβ subunit is expressed in excess. Dimerisation is obligatory for hormonal activity, as the individual hCG subunits are devoid of bioactivity (Narayan *et al.* 2002). Consequently, the sexual dimorphism observed in the secreted bioactive forms of hCG would be attributed to a differential sex steroid feedback regulation of common α-subunit expression at the hypothalamic–pituitary level, as was observed elsewhere (Risma *et al.* 1995).

The female phenotype of the hCGβ + mice presented with precocious puberty, disrupted oestrous cycles and infertility due to ovarian and uterine defects (Rulli *et al.* 2002). The ovaries appeared with occasional haemorrhagic cysts and looked massively luteinised, resembling luteomas. The presence of luteinised cells filled with lipid droplets suggested active steroid synthesis as a consequence of direct hCG hyperstimulation. Accordingly, high levels of oestradiol, testosterone and progesterone were produced from early stages of sexual development. An interesting phenotype emerging from this model was the development of pituitary adenomas and malignant mammary gland tumours in older age, which were strictly dependent on ovarian function, since ovariectomy prevented both effects despite persistent high levels of hCG. Female hCG + mice were hyperprolactinaemic and showed lactotroph hyperplasia followed by development of prolactinomas, probably due to overexposure to oestrogens during peripuberty, followed by persistently elevated levels of androgens as a source of locally produced oestrogens. Due to its luteotrophic properties (Freeman *et al.* 2000), the high prolactin level produced by the hyperplastic/tumorigenic pituitary may help to maintain the luteinised ovary in an active state of progesterone overproduction. In addition, the role of prolactin in mammary gland development and tumorigenesis has been established (Clevenger *et al.* 2003). Consequently, the indirectly orchestrated high hCG production, along with increased oestrogen, progesterone and prolactin levels, thus brought about mammary gland proliferation and differentiation of the hCGβ + mice. The response originally resembled the lactating state of the gland, but was subsequently followed by the appearance of adenocarcinoma with metastatic properties in older age. Due to its complex and multistep hormonal dysregulation, this is a good animal model for the understanding of the hormone-dependent pathogenesis of the pituitary and mammary gland tumours.

The hCGβ + males were fertile and showed only a mild phenotype, in agreement with their moderately increased levels of bioactive hCG. These mice had smaller testes in the face of full spermatogenesis and normal sperm quality, thus failing to demonstrate adverse effects of hCG on male fertility (Rulli *et al.* 2003).

Besides the hCGβ + mouse with rather mild hormonal aberration, our interest was to achieve a mouse model that maintains higher levels of hCG, in order to analyse the consequences of clearly exaggerated response to hCG.

We therefore generated a double transgenic mouse model harbouring both the common α- and hCGβ-subunit transgenes under the same ubiquitin promoter (hCGαβ + mice), by crossbreeding the hCGβ + mice with another transgenic mouse line overexpressing α-subunit under the same promoter. These double transgenic mice are able to co-express both subunits in excess in different tissues, and to produce efficiently the dimeric form of hCG, reaching as high as 2000-fold levels, in terms of biological activity in the circulation, in both males and females (Rulli *et al.* 2003, SB Rulli, P Ahtiainen, M Poutanen and I Huhtaniemi, unpublished observations).

The hCGα-subunit overexpressing mice were normal and fertile, confirming the absence of biological effects of free gonadotrophin subunits. In contrast to the single hCGβ + model, the double hCGαβ + males were infertile and their reproductive organs were severely altered (Rulli *et al.* 2003). The first series of studies, performed in adulthood, showed smaller testes, enlarged seminal vesicles and prostate, dilated vasa deferentia and urinary bladder, as well as kidney defects. Testicular steroidogenesis was enhanced despite a clear down-regulation of LH/hCGR expression. In agreement with previous studies, based on long-term LH/hCG treatments *in vivo* (Risbridger *et al.* 1982, Gaytan *et al.* 1994), these mice developed focal Leydig cell hyperplasia/hypertrophy, but failed to promote testicular tumours. This was intriguing, since in humans, a specific activating mutation of the LHR ($Asp^{578}His$) is associated with Leydig cell adenomas (Liu *et al.* 1999, Richter-Unruh *et al.* 2002). Interestingly, recent studies in young hCGαβ + mice demonstrated postnatally clear Leydig cell adenomas of foetal Leydig cell origin, but these tumours disappeared at puberty (Ahtiainen *et al.* 2005). Whether the activation of alternative intracellular signalling pathways of the LHR, besides cAMP (Liu *et al.* 1999), explain the early Leydig cell tumorigenesis remains to be elucidated. An abnormal function of the accessory sex organs and the lower urinary tract was also evident in these males. Functional obstruction of vasa deferentia due to overproduction of secretory fluids or impaired emptying of the glands is the likely cause for the male infertility observed in this model. Progressive degenerative changes in the seminiferous epithelium and epididymides are associated with obstruction through a backpressure effect, and most of such changes may be due to severe steroid imbalance, in concordance with the male phenotype of oestrogen receptor-α knockout mice with elevated androgen levels (Eddy *et al.* 1996).

Interestingly, our recent investigations demonstrated that hCGαβ + female mice with overexpression of excessive levels of hCG developed ovarian tumours that are phenocopies of human teratomas (Huhtaniemi, *et al.* 2005, SB Rulli, P Ahtiainen, M Poutanen and I Huhtaniemi, unpublished observations). This novel finding strengthens the applicability of this model for studying human diseases, since no other model has previously shown any linkage between ovarian teratomas and gonadotrophin action. It is well known that these tumours derive from parthenogenetically activated oocytes within the ovary (Mutter 1997, Ulbright 2004). The mechanism that triggers the initial steps of tumour formation in our model is currently under investigation.

More recently, another transgenic model for hCG has been reported, where either one or both subunits of hCG were overexpressed using the mouse metallothionein promoter (Matzuk *et al.* 2003). In this model, hCGβ overexpressing females were infertile and progressively developed cystic ovaries, whereas males were infertile despite no discernible phenotype. In contrast, transgenic male mice co-expressing hCGα- and hCGβ-subunits showed multiple reproductive defects resembling those found in our previous model, such as infertility, Leydig cell hyperplasia, increased testosterone, reduced testis size and enlarged seminal vesicles. Double-transgenic females were infertile, had elevated oestradiol levels, and developed cystic ovaries with thecal layer enlargement and stromal cell proliferation, and degenerating kidneys (Matzuk *et al.* 2003). No evidence for tumorigenesis in gonadal or extragonadal tissue was reported in this model.

A transgenic mouse model expressing a constitutively active yoked hormone–receptor complex was recently generated, where hCG was covalently linked to the N-terminus of the rat LH receptor in a fusion protein (Meehan *et al.* 2005). Males exhibited prepubertal increases in testosterone levels and seminal vesicle weights, and decreases in serum FSH, LH, testis weight, and the size of the seminiferous tubules. Females presented with precocious sexual development and progressive ovarian lesions, from enhanced follicular development to degenerating follicles and haemorrhagic cysts.

Taken together, these studies indicate that chronically elevated hCG leads to multiple gonadal and extragonadal defects in males and females, including ovarian and testicular tumours as the primary effect, whereas the alterations found in the pituitary and mammary glands are due to secondary effects of the aberrant gonadal function. The distinct phenotypes emerging from the existing mouse models overexpressing hCG/LH *in vivo* may be related to the level of hCG/LH production, age, characteristics of the transgene expression, and the genetic background. However, the phenotypic similarities among the different models further emphasise the role of gonadotrophin action in reproductive pathophysiology.

Genetically modified mouse models with secondarily altered gonadotrophin secretion

Gonadotrophin synthesis and secretion is modulated by different steroidal and non-steroidal ovarian factors. Consequently, genetic mouse models directed to those factors can induce secondary gonadotrophin imbalance and display disturbed reproductive phenotypes similar to those found in gonadotrophin overexpressing mice. One typical example is the knockout mouse model for

oestrogen receptor α (αERKO), which has elevated LH levels due to the loss of oestrogen-mediated feedback regulation of the hypothalamic–pituitary–gonadal axis, and shows some similarities with the phenotypes of gonadotrophin overexpressing models (Schomberg *et al.* 1999). It was demonstrated that high LH levels are responsible for the defective ovarian phenotype in αERKO mice, since increased LH and polycystic follicle development are prevented by GnRH antagonist treatment (Couse *et al.* 1999*a*).

αERKO male mice are infertile due to abnormal fluid reabsortion in the epididymis, leading to disrupted spermatogenesis and seminiferous tubule organisation through a backpressure effect (Eddy *et al.* 1996). These mice exhibit elevated serum testosterone levels, but LH and FSH levels are not significantly different from those of wild-type males. These results are in line with the male phenotype of hCGαβ + mice (Rulli *et al.* 2003), in which elevated androgen levels may be responsible for the defective phenotype of the male reproductive system.

The aromatase knockout model (ArKO) is characterised by lack of oestrogen production, which induces testosterone, LH and FSH levels in circulation. Similarly to αERKO, the ArKO mice are infertile due to disrupted folliculogenesis and failure to ovulate, and develop haemorrhagic cystic follicles (Britt *et al.* 2001). The ovarian phenotype of the ArKO mouse was then attributed to the altered hormonal milieu, where the conversion of androgens to oestrogens is blocked in the presence of elevated gonadotrophins.

Concluding remarks

Application of new technologies for the development of experimental animal models together with the characterization of human phenotypes with genetic defects have provided a great deal of novel information on less well known mechanisms involved in pathological effects of gonadotrophin function in both sexes. The different transgenic mouse models with gonadotrophin overexpression available in the literature are increasing in number and reveal marked differences and similarities in their phenotypes (Table 1). As a consensus from most of the models, female fertility and ovarian physiology are particularly vulnerable to alterations of gonadotrophic action, mainly manifested by different levels of hormone profile disruption, and development of cystic and/or tumorigenic ovaries as the primary effect. In the most severe cases, secondary gonad-dependent phenotypes, such as urinary tract disturbances and extra-gonadal tumour development (mammary and pituitary gland tumours, adrenal lesions) are also observed. The male phenotypes in the different models appear much milder in terms of fertility and gonadal tumour development. However, discrete phenotypes of the male reproductive tract also occur.

Taken together, these 'lessons' from transgenic mice provide important evidence for the impact of gonadotrophin hypersecretion on the gonadal function and its relation with human pathologies. The novel findings clearly advance our knowledge beyond that obtained previously in experiments using various gondotrophin treatments or ablations. Since many aspects of reproductive pathophysiology are not completely understood, and new questions are constantly arising, additional studies providing more detailed information are needed in the future.

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