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Review

Phenotypic characterisation of mice with exaggerated and missing LH/hCG action

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Abstract

In order to study the physiology and pathophysiology of gonadotrophin action, we have produced transgenic (TG) mice overexpressing human chorionic gonadotrophin (hCG) α and β subunits (hCG+ mice) and knockout (KO) mice for the luteinising hormone receptor (LHR; LuRKO mice). The two extremes in LH function, i.e. strong LH/hCG stimulation and total blockade of this action, confirm numerous earlier concepts about LH function, but they also reveal new aspects about gonadal function during excessive LH production and in the absence of this trophic stimulus. The purpose of this review is to summarise the key findings on these two genetically modified mouse models.

Keywords: Luteinising hormone; Luteinising hormone receptor; Transgenic; Knockout; Ovary; Testis; Pituitary gland; Tumorigenesis; Luteoma; Prolactinoma

Contents

1. Introduction

The targeted modification of gene structures allows the production of both gain-of-function and loss-of-function mutations in experimental animals, usually mice. The former approach uses the TG technique with pronucleus injection of DNA constructs that, depending on the promoter sequence, bring about targeted or general overexpression of a new gene. The latter approach uses targeted disruption of specific endogenous genes by homologous recombination in embryonic stem cells. Gene disruption can also be achieved in spatio-temporal manner, using for instance the cre/loxP techniques. The genetically modified mice created are extremely useful in studies on molecular mechanisms involved in exaggerated or suppressed function of specific genes, and they often provide accurate phenocopies of respective mutations or functional aberrations in humans, thus

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Table 1
Genetically modified mouse models for gain (transgenic) and loss (knockout) of LH/hCG function

	Phenotype	References
Gain-of-function models		
hCGβ promoter/hCGβ cluster	No phenotype	Strauss et al. (1994)
bLHα promoter/bLHβ-CTP	Males fertile; females polycystic ovaries, granulose/theca cell tumours, pituitary adenomas, mammary tumours	Risma et al. (1995)
Ubiquitin C promoter/hCGβ	Males fertile; females infertile, ovaries luteinised, prolactinomas, malignant mammary adenocarcinomas	Rulli et al. (2002)
Ubiquitin promoter/hCGαβ	Males fertile, fetal Leydig cell adenomas, urethral and kidney defects; females infertile, ovarian luteomas and teratomas	Rulli et al. (2003)
Metallothionein-1 promoter/hCGβ	Males and females infertile; cystic and haemorrhagic ovaries	Matzuk et al. (2003)
Metallothionein-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)
Loss-of-function models		
Common-α subunit	Absence of LH, FSH and TSH, normal sexual differentiation in utero, total lack of postnatal sexual development, hypogonadism, hypothyroidism	Kendall et al. (1995)
LHβ	Males infertile, normal prenatal masculisation, cryptorchidism, hypoandrogenism, no pubertal development, Leydig cell hypoplasia; females delayed puberty, infertile, anovulation	Ma et al. (2004)
LH receptor	Identical phenotype with LHβ knockout	Zhang et al. (2001a,b), Lei et al. (2001)

allowing mechanistic studies of molecular pathogenesis of these conditions.

As concerns gonadotrophin function, mouse models for most permutations of gain-of-function and loss-of-function mutations of the key genes, i.e. those for the two gonadotrophin subunits and their two receptors, have been produced. The ligand and receptor genes have been disrupted by KO techniques (Abel et al., 2000; Dierich et al., 1998; Kendall et al., 1995; Kumar et al., 1997; Lei et al., 2001; Ma et al., 2004; Matzuk et al., 2003; Zhang et al., 2001a), and there are several TG mouse lines overexpressing gonadotrophin subunits and mimicking gonadotrophic hyperstimulation (Kumar et al., 1999; Risma et al., 1995; Rulli et al., 2002). The only class of human mutations not yet having mouse models are the activating point mutations of the gonadotrophin receptors that either bring about constitutive signal transduction, or alter the signaling mechanisms or ligand specificity of the receptors.

We have produced genetically modified mice that overexpress α- and β-subunits of the LH agonist human chorionic gonadotrophin (hCG; hCG+ mice) (Rulli et al., 2002, 2003) and have disrupted LHR gene (LuRKO mice) (Zhang et al., 2001a). They simulate conditions at the two extreme ends of LH action from total inactivation to overproduction and thus elucidate many aspects of the pathophysiology of LH/LHR function. The purpose of this article is to review the main findings on these two experimental mouse models.

2. hCG overexpressing transgenic mice

TG mice overexpressing hCG were created by conventional pronucleus injection. hCGβ subunit (hCGβ+ mice) was driven by the ubiquitously expressed human ubiquitin C promoter, and dimerisation with the endogenous common α-subunit occurred only in the pituitary gland, the physiological site of expression of this gene. This led to 40-fold increase in LH/hCG bioactivity in female mice, but only 3- to 4-fold increase in males (Rulli et al., 2002, 2003). The elevation of dimeric α-subunit/hCGβ was modest because of restricted expression of the former. We then created another TG mouse line (hCGα+) expressing the glycoprotein hormone common α-subunit under the same ubiquitin C promoter and intercrossed them with the hCGβ+ mice. We achieved a double-TG model, which expressed both subunits ubiquitously and this resulted in very high, more than 1000-fold, increase in LH/hCG bioactivity both in males and in females (Ahtiainen et al., 2005; Rulli et al., 2003).

Two other LH/hCG overexpressing TG mouse models have been published, one expressing the bovine LHβ/hCGβ C-terminal peptide (CTP) fusion protein under the bovine common-α subunit promoter (Risma et al., 1995), the other expressing hCG subunits under the mouse metallothionein-1 promoter (Matzuk et al., 2003). The phenotypes of the different models are in principle very similar, although some quantitative and qualitative differences do exit in the responses because of different levels of transgene expression and different mouse strain used (Table 1). We describe below in more detail the phenotype of the ubiquitin C/hCGβ and hCGα/β overexpression mice.

2.1. Female phenotype

The phenotype of the female hCGβ+ mice was reported previously (Rulli et al., 2002). These females presented with precocious puberty followed by disruptions of the oestrous cycle and infertility. Oestradiol levels were about 3-fold elevated at the age of 1 month, but indistinguishable from controls thereafter. There were occasional hemorrhagic cysts and marked luteinisation in the ovaries as consequence of the strong hCG stimulation (Fig. 1). For the same reason, these females produced high levels of progesterone, which remained high, in the micromolar range, for the rest of their life. Interestingly, also

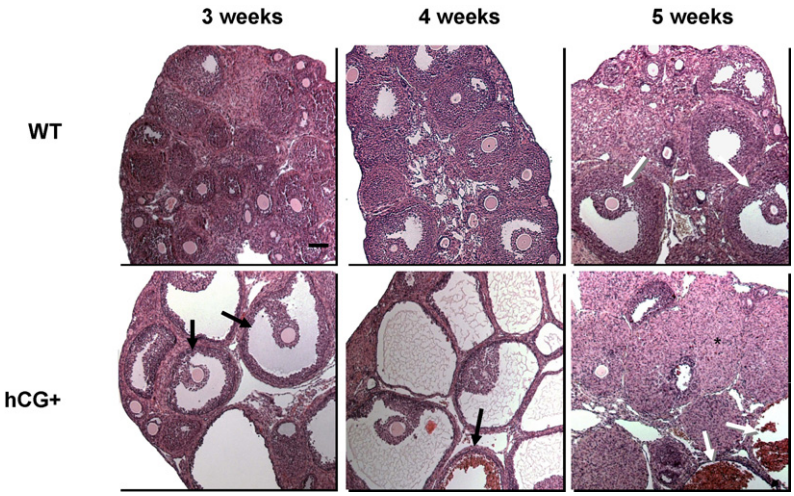

Fig. 1. Ovarian histology of 3-, 4- and 5-week-old WT and hCG+ mice. The appearance of ovaries was similar in single-TG hCGβ+ and double-TG hCG+ mice. Antral follicles (arrows) are the most mature form in WT ovaries at the age of 5 weeks. We can see in the TG ovaries already at 3 weeks premature formation of antral follicles with few layers of granulosa cells (arrows). At 4 weeks, there are multiple haemorrhagic (arrow) and fluid-filled cysts, and at 5 weeks there is evidence of strong luteinisation (asterisk) and haemorrhagic cysts (arrow).

testosterone production of the hCGβ+ females was increased about 5-fold as compared with WT controls. Many of the abnormalities observed in the hCGβ+ females contributed to their infertility, but most probably their anovulation was due to the tonic hCG stimulation and/or high androgen production.

Several extragonadal phenotypes were also observed in the hCGβ+ females (Rulli et al., 2002). The mice developed pituitary hyperplasia at the age of 2 months, followed by gradual growth of pituitaries to macroprolactinomas at the age of 6 months. It is known that oestradiol promotes lactotroph hyperplasia, and the high oestradiol levels observed during peripuberty in the hCGβ+ females could be responsible for the original lactotroph growth. However, at the age of 2 months, the oestradiol levels returned back to normal, indicating that something else than oestradiol may have been involved in the subsequent development of macroprolactinomas. One interesting candidate is progesterone, which displayed concentration rises in the hCGβ+ females up to 100-fold higher than in WT females. We have shown that ovariectomy prevents the formation of prolactinomas indicating that factor/s responsible for this are derived from ovaries.

The old hCGβ+ females developed mammary gland adenocarcinomas at the age of 9–12 months. The complex hormonal changes brought about by hCG stimulation preceded this event which was also shown to be ovary dependent because ovariectomy prevented the tumorigenesis. At the time of appearance of the mammary carcinomas prolactin (PRL) secretion was already 500–1000-fold increased in the hCGβ+ females. This endocrine aberration was most probably crucial for the mammary gland tumorigenesis, but most possibly also high hCG and progesterone and peripubertally increased oestradiol affected the tumor development. More studies are needed to confirm the role of each hormone in the mammary gland tumorigenesis.

We are currently analysing the phenotype of females overexpressing both common-α and hCGβ subunits (hCG+ mice). Despite the very highly elevated (>1000-fold) LH/hCG bioactivity in their circulation, these females present qualitatively with very similar phenotypes as the hCGβ+ females (Fig. 1 and Table 1). However, because of the much higher hCG-production their phenotypes were even more pronounced. These females are also infertile, their hormonal profile is similar to the hCGβ+ females, and they also develop lactotroph hyperplasia in their pituitary glands. The only clear difference between these two mouse models is that hCG+ females developed ovarian teratomas at the age of approximately 2 months (Rulli et al., unpublished). We believe that formation of teratomas is driven by the high hCG concentration, because otherwise the hormonal milieu is similar between these two mouse models. We are currently addressing the molecular mechanisms behind the teratoma formation.

2.2. Male phenotype

The phenotype of the hCGβ+ males was very mild as a consequence of their only slightly increased (3–5 fold) LH/hCG bioactivity (Rulli et al., 2003). These males were completely fertile with full and normal spermatogenesis. The only phenotypic features observed were the slightly decreased serum FSH levels and the consequent slight reduction in testis size, as compared with WT males.

More pronounced phenotypes were observed in males overexpressing both hCG subunits (hCG+ males) (Rulli et al., 2003). These mice produced 1000-fold excess of bioactive hCG/LH. They were infertile and severe changes were observed in their reproductive organs (Fig. 2). Most if not all the phenotypic changes observed in these males were caused either directly or indirectly by the 30-fold increased testosterone concentration. The high testosterone apparently suppressed FSH secretion and consequently the testis sizes of the hCG+ males were 50% reduced even though spermatogenesis appeared histologically completely normal. High testosterone was also responsible for the enlargement of seminal vesicles and prostate (Fig. 2).

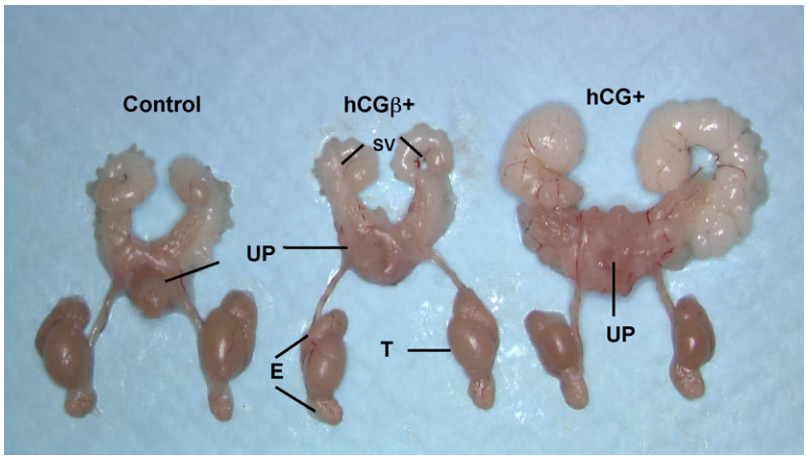

Fig. 2. Macroscopic appearance of genital structures in WT, hCGβ+ and hCG+ mice. No major differences were found between the WT and hCGβ+ mice, whereas the seminal vesicles (SV) and utetroprostatic block (UP) were enlarged, and testes (T) reduced in size in hCG+ mice. E, epididymis.

Signs of urinary tract obstruction were also seen, and the hCG+ males presented with dilated urinary bladder, vasa deferentia and ureters as well as some signs of hydronephrosis. We were unable to detect any anatomical obstruction at the prostatic level, so the voiding/smooth muscle function of the urinary tract was apparently impaired in the hCG+ males. Because of the high testosterone, the males behaved aggressively and were unable to mate normally with females. Whether the infertility was totally due to abnormal behaviour or to anatomical or functional obstruction of the ejaculatory ducts, is not yet known. The most surprising finding was that the adult hCG+ males failed to develop any testicular tumors despite the excessive hCG stimulation of Leydig cells, which was expected because in man a specific activating *LHR* mutation ($Asp^{578}His$) is associated with Leydig cell adenomas (Liu et al., 1999). Altogether, these observation prompted the conclusion that the protective mechanisms against pathological effects of LH/hCG hypersecretion in male mice are much more efficient than in females.

To see how the pubertal development of male mice was affected by high hCG levels, we analysed hCG+ mice between the ages of 5–60 days (Ahtiainen et al., 2005). To our surprise, we were not able to detect any signs of precocious puberty despite highly elevated circulating testosterone. The body size, timing of the balano-preputial separation and onset of spermatogenesis were indistinguishable in the hCG+ and WT males. This finding was surprising and suggested that the onset of pubertal maturation in mice is already at its minumum in WT males, or that it is triggered by some other factor than hCG or testosterone. Histological analysis of the testes showed that prepubertal hCG+ mice had large Leydig cell nodules, which could be classified as adenomas (Fig. 3). These adenomas reached their maximum size at the age of 10 days and regressed between the ages of 21 and 60 days. The expression pattern of fetal and adult Leydig cell markers suggested that the adenomas observed in the hCG+ males were derived from fetal Leydig cells, and these adenomatous fetal Leydig cells regressed according to their normal lifespan, explaining the disappearance and the absence of Leydig cell adenomas in adult age (Ahtiainen et al., 2005). Moreover, the adult

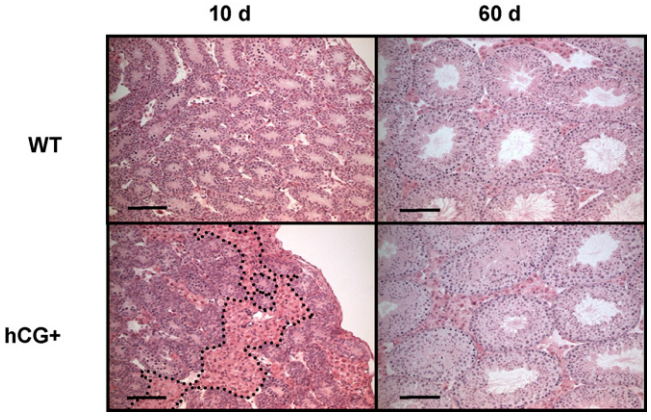

Fig. 3. Testicular histology in 10 and 60-day old WT and hCG+ mice. A large Leydig cell adenoma (dotted line) is present in the 10-day old hCG+ testis, but no apparent differences are seen between the hCG+ and WT testes at 60 days. Modified from Ahtiainen et al. (2005).

Leydig cells seemed to be resistant to gonadotrophin-stimulated formation of adenomas.

Taken together, it was not a priori expected that high hCG stimulation could bring about such a pronounced phenotype in females but only quite slight response in males. The phenotype of man with activating *LHR* mutation is totally opposite: men develop precocious puberty and testicular tumors in some cases, whereas female carriers of similar mutations show no apparent phenotype (Themmen and Huhtaniemi, 2000).

3. *LHR* knockout mice

Two KO models for *LHR* were published in 2001, by Zhang et al. (2001a) and Lei et al. (2001) (Table 1). The former model was created by targeted disruption of the long 11th exon of *LHR*, encoding the transmembrane and intracellular domains of the receptor, the latter model by disruption of the proximal part of the *LHR* promoter region and exon 1. All evidence demonstrates that both models produce complete elimination of functional LHR in the (−/−) mice. In principle the phenotypes observed in the two

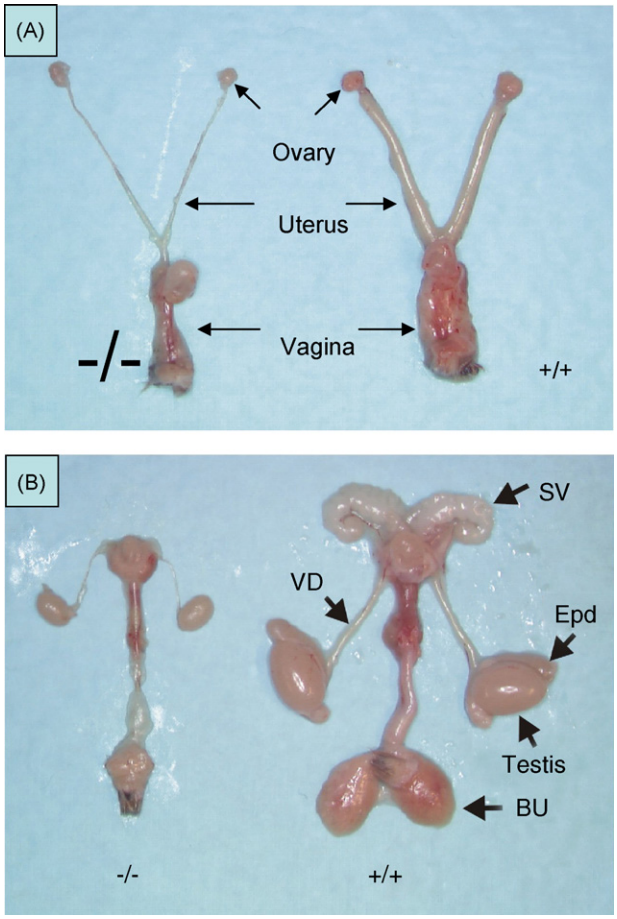

Fig. 4. Macroscopic appearance of the genital structures of WT (+/+) and LuRKO (−/−) female (Panel A) and male (Panel B) mice. VD, vas deferens; SV, seminal vesicle; Epd, epididymis; BU, bulbourethral gland. Modified from Zhang et al. (2001a,b).

models are identical, although the two laboratories that produced the mice tend to interpret their findings somewhat differently, in particular as concerns the evidence for or against the functional significance of extragonadal LH/hCG action (Chudgar et al., 2005; Lin et al., 2005; Pakarainen et al., 2005b; Rao, 2001).

3.1. Female phenotype

Most phenotypic features of the female LuRKO mice were expected and in line with the existing knowledge about LH function (Zhang et al., 2001a). The intrauterine sex differentiation of the females was normal, as is expected because the ovarian *LHR* expression does not start until a few days after birth and the fetal ovaries do not have significant endocrine activity. In adult age, the female LuRKO mice present with anovulatory infertility and hypo-oestrogenism, as demonstrated by their poorly developed accessory sex glands, including thin uteri (Fig. 4). There is a total lack of follicular maturation beyond the antral stage which indicates, besides the essential role of LH in ovulation, that the final maturation of follicles from antral to preovulatory stage is also dependent on LH stimulation.

Earlier studies on hypophysectomised rats and mice have indicated that ovulation can be induced with recombinant FSH without any LH activity (Galway et al., 1990; Tapanainen et al., 1993). Besides the regulation of follicular maturation, FSH may thus have a role in ovulation and luteinisation, because ovulation can be triggered by high dose of FSH without concomitant LH surge. Although these findings appear convincing, it still remains uncertain whether the apparently FSH dependent ovulation is mediated purely via FSHR activation, or whether some level of LHR stimulation is also needed. Permissive effect of a low degree of constitutive LHR activation may be needed for the unexpected FSH dependent ovulation. Furthermore, evidence for a role of the epidermal growth factor (EGF) family members, amphiregulin, epiregulin and betacellulin, as mediators of the LH action in follicle maturation, has been recently demonstrated (Park et al., 2004). These proteins have been shown to mediate LH actions by acting as paracrine mediators between mural granulosa and cumulus cells, and to be necessary for the formation of cumulus–oocyte complexes and oocyte maturation.

To revisit the question of FSH stimulated ovulation, we assessed in LuRKO mice the progression of folliculogenesis and induction of ovulation by hCG and recombinant human FSH treatments (Pakarainen et al., 2005b). As expected, hCG had no effect in LuRKO mice, and the results provided clear evidence that follicular development and ovulation could not be induced by high doses of FSH in the absence of functional LHR. Ovarian histology indicated that follicular maturation did not advance in LuRKO mice beyond the antral follicle stage, whether or not they were treated with FSH. Neither were ovulations detected in the LuRKO ovaries after any of the gonadotrophin treatments. The ovarian resistance to FSH treatment in the absence of LHR was confirmed by real-time RT-PCR and immunohistochemical analyses of a number of gonadotrophin-dependent genes, which only responded to the treatments in WT mice. Neither did oestradiol priming prior to gonadotrophin stimulations alter the negative outcome. Hence, this study showed that LHR expression is essential, besides ovulation, also for the final follicular maturation from antral to preovulatory stage. The discrepancy between the findings in hypophysectomised and LuRKO mice in their FSH responsiveness is puzzling and not readily explicable. A very recent finding offers an intriguing explanation (Urizar et al., 2005). These authors found that when FSH and LH receptors were expressed in the same cell they were able to form heterodimers. Because luteinising granulosa cells do express both receptors simultaneously before ovulation, there is a possibility that such heterodimers could form during the preovulatory period, have a physiological function, and explain why FSH stimulation can induce ovulation in hypophysectomised but not in LuRKO mice. Gonadotrophin receptor expression still persists in gonads after hypophysectomy, and ligand-bound FSHR could form functional dimers with non-liganded LHR during FSH treatment, but this is not possible in LuRKO ovaries. It would be intriguing to carry out a similar experiment with FSH treatment in *LHβ* KO mice (Ma et al., 2004).

Quite unexpectedly, a number of studies have recently reported that LH/hCG receptors are expressed in a variety of extragonadal tissues, including the uterus, oviduct, cervix, placenta, mammary gland, sperm, certain areas of the brain, and many others (Fields and Shemesh, 2004; Filicori et al., 2005;

	No. of mice bred	Vaginal plugs	Litters born	No. of pups/litter
LuRKO + WT ovaries	16	95 %	9 (56 %)	4.2 ± 2.4
WT + WT ovaries	20	95 %	16 (80 %)	4.4 ± 2.3
WT, without operatio	10	100 %	10 (100 %)	6.0 ± 2.4

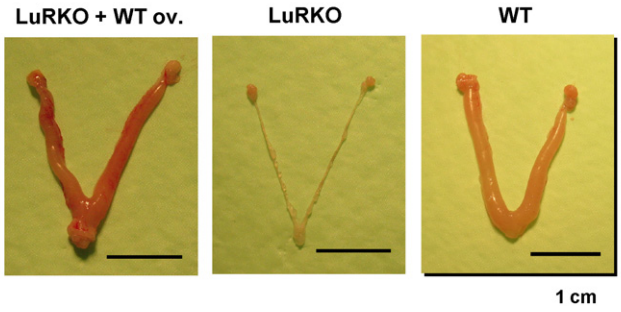

Fig. 5. Breeding data of WT and LuRKO mice transplanted orthotopically with WT ovarian tissue (upper part). The macroscopic appearance of uterus is shown in the lower part, a LuRKO mouse transplanted with WT ovary on the left, a LuRKO control mouse in the middle, and a WT mouse on the right. Modified from Pakarainen et al. (2005a).

Rao, 2001; Shemesh, 2001; Zhang et al., 2001b). Many of these findings were made on human tissues, but similar observations have also been reported on a number of other mammalian species, including baboons, bovines, pigs, rats, and mice (Apaja et al., 2004; Fazleabas et al., 1999; Shemesh et al., 2001; Zhang et al., 2001b; Ziecik et al., 1986). On the basis of these observations, a shift in the old paradigm that gonadotrophins only have gonadal actions has been proposed (Lin et al., 2005; Rao, 2001).

A number of extragonadal LH/hCG actions have been suggested, including effects on brain development and sexual behaviour, regulation of uterine blood flow, survival of early pregnancy, decidualisation, and regulation of uterine growth and enzyme levels (Filicori et al., 2005). Although many of these effects appear at face value convincing, there are several caveats: (1) often only fragments of the LHR mRNA have been identified using RT-PCR, without evidence for functional full-length message, (2) the size of immunoreactive LHR protein detected by immunoblotting does not always agree with the authentic gonadal receptor, and often heterologous antibodies have been used, (3) most of the data have been obtained in in vitro incubations and cell cultures, and compelling in vivo data are largely missing, (4) human inactivating mutations of *LHR* have only gonadal phenotype, and all extragonadal effects can be explained by gonadal dysfunction, and (5) all extragonadal phenotypes so far observed in LuRKO or LH/hCG overexpressing mice can be explained by altered gonadal function.

To obtain further in vivo evidence about the potential extragonadal LH/hCG effects, we set out to assess the effect of ovarian transplantation on mouse reproductive maturation, fertility, pregnancy and lactation (Pakarainen et al., 2005b). Prepubertal (23-day-old) LuRKO females were orthotopically replaced with pieces of WT ovary, using similarly transplanted WT mice as controls (Fig. 5). Most ovarian transplants attained normal endocrine function in both groups of mice, as demonstrated by normal age at vaginal opening, estrous cycle, and sexual behaviour. Both the LuRKO and WT control mice became repeatedly pregnant (9/16 versus 16/20 after first mating; difference not significant) and delivered litters of similar size, which grew normally after birth, indicating normal lactation. These findings allowed the conclusion that fertility can be fully restored in LuRKO mice by transplantation of WT ovarian tissue. Because the transplant was the only site of functional LHR in these animals, extragonadal LH/hCG action was apparently not necessary for normal sexual maturation, sexual behaviour, maintenance of pregnancy, delivery or lactation. Thus, the findings indicate physiological redundancy for these receptor sites at least in the mouse. The group of Rao (Chudgar et al., 2005), using their *LHR* KO mouse model, were unable to achieve pregnancies after similar orthotopic transplantations of WT ovaries. The reason for the negative outcome remains open, but it may be technical.

Novel information is continuously accumulating about extragonadal effects of gonadotrophins. Whether such effects, usually demonstrated in vitro, have any significance in the physiological context, remains a contentious issue. Our finding that LuRKO mice with orthotopically transplanted WT ovarian tissue gain normal fertility speaks against their physiological importance. We recently observed that direct LH/hCG effects on bone were suggested by the findings on bone densities in female WT, LuRKO and hCG+ mice (Yarram et al., 2003). Bone density was highest in hCG+ mice, intermediate in WT, and lowest in LuRKO mice. However, all differences between the groups disappeared when the mice were gonadectomised, indicating that the gonadotrophin effects on bone were indirect through regulation of ovarian function. However, the presence of low levels of extragonadal LH/hCG receptor is apparently a genuine finding, as well as the several extragonadal effects of these hormones, even though mostly observed in vitro. It is clear that further information is needed before the final answer to this conundrum can be obtained.

3.2. Male phenotype

With respect to the male rat and mouse, many experimental models have shown before that fetal testicular testosterone production, essential for the intrauterine masculinisation, is not dependent on gonadotrophin stimulation (see, e.g. Huhtaniemi, 1994; Kendall et al., 1995; Pakarinen et al., 2002). For this reason, the normal masculinisation of LuRKO mice at birth was expected. The function and histological appearance of the testes in newborn LuRKO were indistinguishable from WT mice. There is plenty of evidence in the rodent, that in the absence of LH action, steroidogenesis of fetal Leydig cells can be maintained by a number of bioactive peptides in paracrine fashion. Such compounds are, for instance PACAP, ANP, VIP and ACTH (El-Gehani et al., 1998a,b, 2000, 2001; O'Shaughnessy et al., 2003). This is at variance with the human, where completely inactivating *LHR* mutations totally block male fetal masculinisation causing pseudohermaphroditism (Themmen and Huhtaniemi, 2000). However, missing pituitary LH secretion can be compensated for by hCG, but if the LHR is defective, mansculinisation does not occur. It is intriguing that the backup mechanism of fetal Leydig cell regulation is so different in the two mammalian species, hCG in humans and non-gonadotrophic paracrine factors in rodents. The postnatal dependence of adult Leydig cell function on pituitary LH is absolute in both species: both men without bioactive LH (Valdes-Socin et al., 2004; Weiss et al., 1992) and mice with *LHβ* or *LHR* KO (Ma et al., 2004; Zhang et al., 2001a) have total lack of postnatal sexual maturation with hypogonadism, cryptorchidism, poorly developed accessory sex glands and spermatogenic arrest at the round spermatid stage (Table 1).

Detailed follow-up of postnatal testicular development in LuRKO mice (Zhang et al., 2004) revealed that the testicular histology of these and control WT mice was similar until about 2 weeks of postnatal life, but in adulthood the former were devoid of mature Leydig cells and showed thin seminiferous tubules with arrested spermatogenesis. Testicular testosterone concentration at birth was indistinguishable between WT and LuRKO testes. When the expression levels of several Leydig cell and steroidogenesis specific genes were followed, they initially showed similar level of expression in WT and LuRKO mice. But after puberty, most of them (such as cytochrome P 450 side chain cleavage, 17α-hydroxylase cytochrome P 450, 17β-hydroxysteroid dehydrogenase [HSD] III, steroidogenic acute regulatory protein [StAR], insulin like factor 3) were expressed at very low level in LuRKO testes in accordance with the lack of growth and maturation of the adult Leydig cell population. The only steroidogenic gene behaving differently was *3β-HSD I*, which was equally highly expressed in adult WT and LuRKO testes. The reason may be that it is expressed already in mesenchymal and peritubular Leydig cell precursors (Haider and Servos, 1998). Hence, these studies confirmed that sufficient fetal Leydig cell steroidogenesis for masculinisation, although responsive to LH stimulation, is not dependent on action of this hormone.

Because the male LuRKO mice are hypogonadal after birth due to the lack of adult Leydig cell differentiation, this model made it possible to study to what extent testosterone treatment alone, when started before puberty, is able to restore the male phenotype (Pakarainen et al., 2005a). High-dose testosterone replacement therapy was initiated in LuRKO mice at the age of weaning (21 days) and continued until the age of 60 or 90 days. The treatment induced in LuRKO mice a male phenotype that was indistinguishable from WT controls, including testicular descent and full spermatogenesis. Hence, testosterone priming before the age of 21 days is not necessary in male LuRKO mice for their subsequent normal sexual maturation. The mice have normal testosterone levels at birth, but become gradually hypoandrogenic after 10 days of age, since the fetal Leydig cell population, active after birth, will not be replaced by steroidogenically active adult Leydig cells (Zhang et al., 2004). Conspicuously, some abnormalities including vigorous inflammation were found in the epididymides and accessory sex glands of the androgen treated LuRKO mice. Also the fertility of these mice was found to be reduced despite apparently normal spermatogenesis and normal sexual behaviour. The mice were found to have low ejaculatory frequency and low sperm counts in cauda epididymidis. Hence, the testosterone treated mice remained subfertile due to combined effect of the above abnormalities on sperm production, transport and deposition. The testosterone dose needed to induce the onset of spermatogenesis had to be so high that it increased serum testosterone level about 10-fold above normal, which could be responsible, though by unknown mechanism, for the accessory sex gland inflammation. Whether it was responsible for the other abnormalities, or whether they were due to the lack of proper androgen priming prepubertally before the onset of the steroid replacement at 21 days of age is not readily apparent.

In another study, we addressed in detail the question on how far spermatogenesis can advance in mice in the absence of LH stimulation, but in the presence of normal FSH level (Zhang et al., 2003). *FSHβ* and *FSHR* KO mice have shown previously that, despite somewhat suppressed testis weight and spermatogenesis, LH/T action alone can maintain spermatogenesis at a level sufficient to maintain normal fertility (Abel et al., 2000; Dierich et al., 1998; Kumar et al., 1997; Zhang et al., 2001a). As young adults the spermatogenesis of LuRKO mice was arrested at the round spermatid stage (Zhang et al., 2001a), in keeping with previous information about the progression of spermatogenesis in the absence of proper androgen production (Plant and Marshall, 2001). However, if we followed the LuRKO males until the age of 12 months, qualitatively full spermatogenesis was observed in their testes with appearance of elongated spermatids of late stage 13–16. Quite unexpectedly, this took place at intratesticular testosterone levels that were only 2% of those in WT controls. However, the mice remained cryptorchid and their epididymides and accessory sex glands were severely underdeveloped, which would have prevented their normal fertility. When the mice were treated with the antiandrogen flutamide, to block the action of the remaining low intratesticular testosterone levels, spermatogenesis stopped at the round spermatid stage, indicating that the very low constitutively produced testosterone level was sufficient to advance postmeiotic spermatogenesis. The normal to slightly elevated FSH levels of the LuRKO mice apparently stim-

ulated spermatognensis to the round spermatid stage, as has been observed in gondotrophin-deficient mice upon FSH supplementation (Allan et al., 2001).

The finding that qualitatively complete spermatogenesis is possible in the absence of the LH-stimulated high intratesticular testosterone production contradicts the current dogma. If extrapolated to humans it may offer the explanation why all men do not reach azoospermia when treated in male contraceptive trials with testosterone to suppress gonadotrophin secretion (Kamischke and Nieschlag, 2004). It may also be that in those men not reaching azoospermia, there is a gonadotrophin-independent component of androgen production that is able to maintain spermatogenesis. Therefore, in order to develop an effective hormonal contraceptive method for man, we should be able to achieve total ablation of intratesticular testosterone levels. How this can be achieved by simultaneously maintaining normal peripheral androgen levels, to prevent the loss of libido and potency, poses a real challenge.

4. Conclusions and future perspectives

The loss and gain-of-function mutations affecting LH/hCG action in genetically modified mice have greatly expanded our knowledge about functions of the regulation of gonadal function (Table 1). Besides the expected effects in line with existing knowledge about LH functions, also novel phenotypic features were found. The most intriguing findings were the maintenance of spermatogenic potential of the testes in the total absence of LH stimulation and the normal high intratesticular testosterone level, the apparent redundancy of extragonadal LHR expression, and the tumorigenic potential of persistently elevated LH/hCG levels in female mice, and the absence of such effects in males. The relevance of these findings to the human remained to be shown in the future. However, the mouse models used demonstrate clearly the great potential of this approach for generating hypotheses for future human research.

The rather robust TG and KO models currently available do not simulate the genetic aberrations detected in human gonadotrophin action, which are usually more subtle inactivating or activating point mutations. Besides simple constitutive inactivation or activation of gonadotrophin action, some of the human mutations bring about qualitative alterations in hormone action. Examples of such situations are the tumorigenic effect of the particular constitutively activating *LHR* mutation, $Asp^{578}His$ (Liu et al., 1999; Richter-Unruh et al., 2002), and the recently discovered *FSHR* mutations that render the receptor responsive to hCG, explaining the molecular pathogenesis of pregnancy-associated ovarian hyperstimulation syndrome (Delbaere et al., 2005). Animal knock-in models for human point mutations are therefore the apparent next step in refining our knowledge about the structure–function relationships in pathologies of gonadotrophin action.

In conclusion, the genetically modified mouse models, including our LuRKO and hCG+ mice, have confirmed many classical concepts of the physiology and pathophysiology of gonadotrophin action. In addition, they have shed light on novel and controversial topics of this field. Lots of research, including improved models simulating more closely the human genetic aberrations, is still needed before a complete picture about the multitude of gonadotrophin actions, their role in various diseases and the therapeutic potentials can be achieved.

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